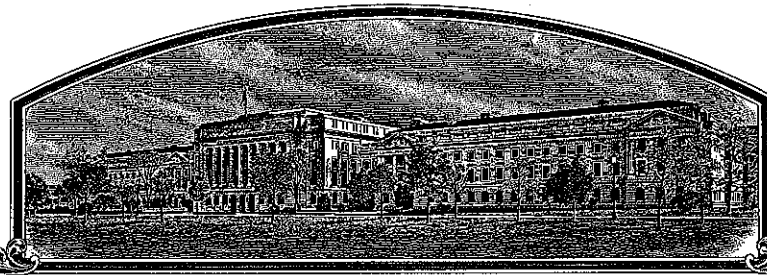


No.

200100227



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

*NDSH Research Foundation*

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLACEMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE FOREGOING PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

POTATO

'Dakota Rose'

*In Testimony Whereof, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of Washington, D.C. this thirtieth day of July, in the year two thousand and eight.*

Attest:

Commissioner  
Plant Variety Protection Office  
Agricultural Marketing Service

Secretary of Agriculture

U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE  
SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE

**APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE**  
(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER <b>NDSU Research Foundation</b>		2. TEMPORARY DESIGNATION OR EXPERIMENTAL NAME <b>ND3574-5R</b>	3. VARIETY NAME <b>'Dakota Rose'</b>
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code, and Country) <b>1735 NDSU Research Park Drive Fargo, ND 58105-5002</b>		5. TELEPHONE (include area code) <b>701-231-8931</b>	FOR OFFICIAL USE ONLY PVPO NUMBER <b>200100227</b> FILING DATE <b>07/23/01</b>
		6. FAX (include area code) <b>701-231-1013</b>	
7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION (corporation, partnership, association, etc.) <b>Corporation - NDSU Research Foundation</b>	8. IF INCORPORATED, GIVE STATE OF INCORPORATION <b>North Dakota</b>	9. DATE OF INCORPORATION <b>May 1989</b>	
10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION. (First person listed will receive all papers) <b>Dr. Asunta (Susie) Thompson NDSU Plant Science Department P.O. Box 5051 Fargo, ND 58105-5002</b> <b>Dale Zetocha Executive Director NDSU Research Foundation PO Box 5014 Fargo, ND 58105-5014</b>			FILING AND EXAMINATION FEES: <b>\$ 2705.00</b> DATE <b>7/3/2001</b> CERTIFICATION FEE: <b>\$ 768.00</b> DATE <b>10/1/07</b>
11. TELEPHONE (Include area code) <b>701-231-7076</b>	12. FAX (Include area code) <b>701-231-7851</b>	13. E-MAIL <b>gary_secor@ndsu.nodak.edu</b>	14. CROP KIND (Common Name) <b>Potato</b>

18. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED (Follow instructions on reverse)		19. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? See Section 83(a) of the Plant Variety Protection Act	
a. <input checked="" type="checkbox"/> Exhibit A. Origin and Breeding History of the Variety b. <input checked="" type="checkbox"/> Exhibit B. Statement of Distinctness c. <input checked="" type="checkbox"/> Exhibit C. Objective Description of Variety d. <input type="checkbox"/> Exhibit D. Additional Description of the Variety (Optional) e. <input checked="" type="checkbox"/> Exhibit E. Statement of the Basis of the Owner's Ownership f. <input checked="" type="checkbox"/> Voucher Sample (2,500 viable untreated seeds or, for tuber propagated varieties, verification that tissue culture will be deposited and maintained in an approved public repository) g. <input checked="" type="checkbox"/> Filing and Examination Fee (\$2,705), made payable to "Treasurer of the United States" (Mail to the Plant Variety Protection Office)		<input type="checkbox"/> YES (If "yes", answer items 20 and 21 below) <input checked="" type="checkbox"/> NO (If "no," go to item 22)	
		20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES? IF YES, WHICH CLASSES? <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED	
		21. DOES THE OWNER SPECIFY THAT THE CLASSES BE LIMITED AS TO NUMBER OF GENERATIONS? IF YES, SPECIFY THE NUMBER 1, 2, 3, etc. <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED (If additional explanation is necessary, please use the space indicated on the reverse.)	
22. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U. S. OR OTHER COUNTRIES? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. (Please use space indicated on reverse.)		23. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT (PLANT BREEDER'S RIGHT OR PATENT)? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO IF YES, GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. (Please use space indicated on reverse.)	

24. The owners declare that a viable sample of basic seed of the variety will be furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Owner(s) is(are) informed that false representation herein can jeopardize protection and result in penalties.

SIGNATURE OF OWNER <b>Dale Zetocha</b>		SIGNATURE OF OWNER	
NAME (Please print or type) <b>Dale Zetocha</b>		NAME (Please print or type)	
CAPACITY OR TITLE <b>Executive Director</b>	DATE <b>6/26/01</b>	CAPACITY OR TITLE	DATE

## INSTRUCTIONS

**GENERAL:** To be effectively filed with the Plant Variety Protection Office (PVPO), **ALL** of the following items must be **received** in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to **reproduce** the variety, or for tuber reproduced varieties verification that a viable (*in the sense that it will reproduce an entire plant*) tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for \$2,705 (\$320 filing fee and \$2,385 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 500, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. **Retain one copy for your files.** All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. **DO NOT** use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$320 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

Plant Variety Protection Office

Telephone: (301) 504-5518

FAX: (301) 504-5291

Homepage: <http://www.ams.usda.gov/science/pvp.htm>

#200100227

## ITEM

- 18a. Give: (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method; (2) the details of subsequent stages of selection and multiplication; (3) evidence of uniformity and stability; and (4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 18b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:
- (1) identify these varieties and state all differences objectively;
  - (2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and
  - (3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 18c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 18d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, etc.
- 18e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
19. If "Yes" is specified (*seed of this variety be sold by variety name only, as a class of certified seed*), the applicant **MAY NOT** reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See Regulations and Rules of Practice, Section 97.103).
21. See Section 83 of the Act for the Contents and Term of Plant Variety Protection.
22. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
23. See Section 5.5 of the Act for instructions on claiming the benefit of an earlier filing date.

**21. CONTINUED FROM FRONT** (Please provide a statement as to the limitation and sequence of generations that may be certified.)

**22. CONTINUED FROM FRONT** (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)

**'Dakota Rose' was released 2/1/01 in the U.S. 'Dakota Rose' was first tested under a Material Transfer Agreement in the U.S. dated 1/13/99 and first tested under a Material Transfer Agreement in Canada dated 4/30/99. Material Transfer Agreements have been used since those times as well and are for testing & evaluation purposes only. No seed sales were authorized.**

**23. CONTINUED FROM FRONT** (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).) **PBR for 'Dakota Rose' has been applied for in Canada (Application No. 99-1733) in July 6, 1999 with protective direction. NorDonna has PVP protection in the U.S.A. issued April 14, 2000, Certificate No. 9600243**

**NOTES:** It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. There is no charge for filing a change of address. The fee for filing a change of ownership or assignment or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

To avoid conflict with other variety names in use, the applicant must check the variety names proposed by contacting: Seed Branch, AMS, USDA, Room 213, Building 306, Beltsville Agricultural Research Center-East, Beltsville, MD 20705. Telephone: (301) 504-8089.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this collection of information is (0581-0055). The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, and marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact the USDA's TARGET Center at 202-720-2600 (voice and TDD). To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

S&T-470 (2-99) designed by the Plant Variety Protection Office with WordPerfect 6.0a. Replaces STD-470 (6-98) which is obsolete.

## EXHIBIT A

## ORIGIN AND BREEDING HISTORY OF 'DAKOTA ROSE'

'Dakota Rose' was evaluated as ND3574-5R and was released by the Agricultural Experiment Station of North Dakota and North Dakota State University (NDSU) on November 17, 2000. 'Dakota Rose' was derived from a cross between North Dakota selections ND1196-2R and NorDonna made March 22, 1987 at NDSU (Figure 1). The clone was initially selected at the Langdon Experiment Station at Langdon, ND in 1988. Early evaluations were conducted at two locations in North Dakota. The initial cross, selection and early testing of 'Dakota Rose' were done under the direction of Dr. Robert Johansen, NDSU (deceased). Advanced testing, seed increase, and commercial evaluation were done by several departments at NDSU, at the USDA-ARS Potato Research Worksite at East Grand Forks, MN, and by several certified seed and commercial producers in North Dakota and Minnesota. Public and private cooperators throughout the United States also provided assistance. Breeder's seed was produced at the Horticultural Research Farm, Absaraka, ND and Agronomy Seed Farm, Casselton, ND. The North Dakota State Seed Department and cooperative certified seed producers under the guidance of the NDSU potato breeding program and the NDSU Development Foundation made subsequent increases. Dakota Rose was widely evaluated in replicated trials at 11 locations in eight years, and in regional trials at 10 North American sites (north central U.S. and Canadian provinces) in 1999 and 2000.

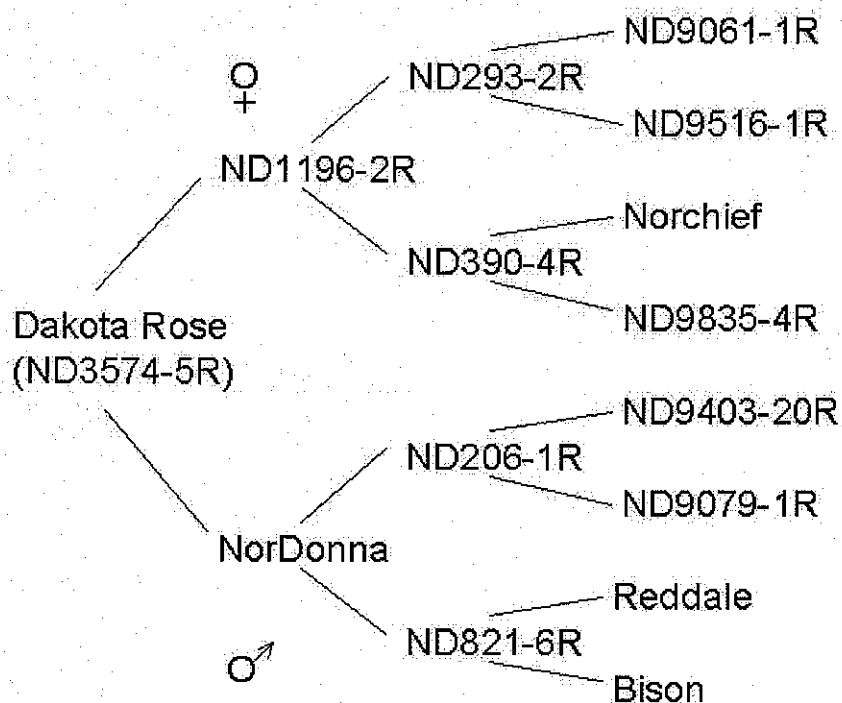
The cultivars NorDonna, Norchief, Bison, and Reddale are in the pedigree of Dakota Rose (Johansen et al. 1969, 1977; F. Lauer, University of Minnesota, unpublished; Novy et al. 1997). NorDonna is a dark red-skinned cultivar with wide adaptation that exhibits few internal defects (Novy et al. 1997). Attributes of Bison include the bright red skin color and very smooth tuber conformation. Yields of Bison are excellent despite the small vine and maturity is medium, between Norland (early) and Red Pontiac (late) (Johansen et al. 1977). At its time of release, Norchief provided an alternative cultivar choice with better red skin color, over commercially available cultivars such as Norland and Red Pontiac (Johansen et al. 1957, 1969). Reddale, a 1984 release from Minnesota (F. Lauer, University of Minnesota, unpublished), was notable for its *Verticillium* wilt (*Verticillium albo-atrum* and *V. dahliae*) resistance; however, commercial production was minimal due to the pale-red color of its skin. Dakota Rose combines many of the attributes of these cultivars including wide adaptability, bright red skin color, smooth tuber type, excellent yields, and relatively few defects have been noted during evaluation. Selection criteria used by the NDSU potato breeding program for the development of 'Dakota Rose' were based on morphological, physiological, and sensory performance.

Since its selection in 1988, 'Dakota Rose' has been asexually propagated via tubers as well as micro-propagated plantlets. During 14 years of evaluation, there have been no reports of variants arising from 'Dakota Rose', indicating it is a stable genotype with uniform morphology.

## Literature Cited

- Johansen, R.H., B. Farnsworth, J.E. Huguelet, D.C. Nelson, and E.P. Lana. 1977. Bison, a new red skinned potato variety. *Am Potato J* 54:189-193.
- Johansen, R.H., J.T. Schulz, and J.E. Huguelet. 1969. Norchief, a new smooth type, high total solids, red-skinned potato variety. *Am Potato J* 46:298-301.
- Johansen, R.H., N. Sander, W.G. Hoyman and E.P. Lana. 1957. Norland, a new red skinned potato variety with early maturity and moderate resistance to common scab. *Am. Potato J* 36:12-15.
- Novy, R.G., R.H. Johansen, G.A. Secor, B.L. Farnsworth, J.H. Lorenzen, N.C. Gudmestad, and E.T. Holm. 1997. NorDonna, a red skinned potato cultivar with wide adaptability. *Am Potato J* 74:31-37.

FIGURE 1. Pedigree of Dakota Rose.



Revised: February 2007

## EXHIBIT B STATEMENT OF DISTINCTNESS

The primary features of Dakota Rose that make it uniquely different from other red potato cultivars, such as Chieftain and Red Pontiac are its bright red skin color and exceptionally smooth skin, in addition to its oblong shape. It is somewhat later in maturity than Red Norland (Thompson et al. 2006), yet earlier than Chieftain and Red Pontiac.

Dakota Rose can be distinguished from other red cultivars, including Chieftain and Red Pontiac, based upon a combination of vine, leaf, flower, and tuber characteristics. Dakota Rose is most similar to and closely resembles Chieftain (Table 1) and is also compared to Red Pontiac, due to its commercial popularity. Dakota Rose can be distinguished from Red Pontiac based on tubers because Red Pontiac has pale red skin, somewhat flakey, and deep eyes, while Dakota Rose has smooth and bright red skin, with very shallow eyes. Tubers of Dakota Rose are more similar to Chieftain. Both have brighter skin color and smoother type than Red Pontiac. The tuber size profile for Dakota Rose tends to be more similar to Chieftain, than to Red Pontiac which is much larger. Vine characteristics of Dakota Rose are also more similar to Chieftain; both have medium green color (146A; Royal Horticultural Society Colour Chart (RHSCC)). Anthocyanin pigmentation is stronger in both Dakota Rose and Chieftain and weak for Red Pontiac. The terminal leaflet base shape is also most similar for Dakota Rose and Chieftain (obtuse to cordate). Vine size for Red Pontiac is larger than for Dakota Rose and Chieftain. All are spreading in type. Flower color is red-purple for all, however the color intensity for Dakota Rose and Red Pontiac is darker and more similar (RHSCC 83C and 86C respectively), compared to Chieftain which is pale (RHSCC 85A).

As presented in Exhibit C, isozyme and DNA (SSR) profiles are also unique. Isoelectric focusing (IEF) electrophoresis revealed distinct protein banding patterns or fingerprints for the five cultivars tested, Dakota Rose, NorDonna, Red Norland, Red Pontiac and Chipeta (Thompson et al. 2006).

Seed certification agencies are able to recognize Dakota Rose as a distinct cultivar in the field and are able to distinguish it from other cultivars based on morphological characteristics during visual inspections of fields entered for certification.

Dakota Rose is suitable for the fresh (tablestock) market. Specific gravity is low, averaging 1.069 in non-irrigated production environments and 1.064 in irrigated production locales. Dakota Rose exhibits no notable disease or pest resistance, nor exceptional susceptibility. In trials, hollow heart has been minimal for Dakota Rose, but was occasionally noted in Red Pontiac. Symptom expression of bacterial ring rot (*Clavibacter michiganensis* subsp. *sepedonicus*) is typical for both vines and tubers. Foliar expression, 80 to 90 days after planting in the Red River Valley, included marginal leaf necrosis, leaf rolling, and interveinal chlorosis. Tuber symptoms include periderm cracking, vascular breakdown and cheesy exudate. Dakota Rose exhibits typical foliar symptoms when infected with Potato Virus Y<sup>0</sup>. It possesses some resistance to common scab caused by *Streptomyces scabies*, and to silver scurf caused by *Helminthosporium solani*.

Results from yield trials, in addition to grower experience in North Dakota, Minnesota and the North Central Regional Trials, indicate that skin set for Dakota Rose is often difficult to achieve. Skinning is often noted and an associated occurrence of soft rot in storage. Dormancy is short.

The unique combination of the above characteristics, used to differentiate potato cultivars, make Dakota Rose distinct.

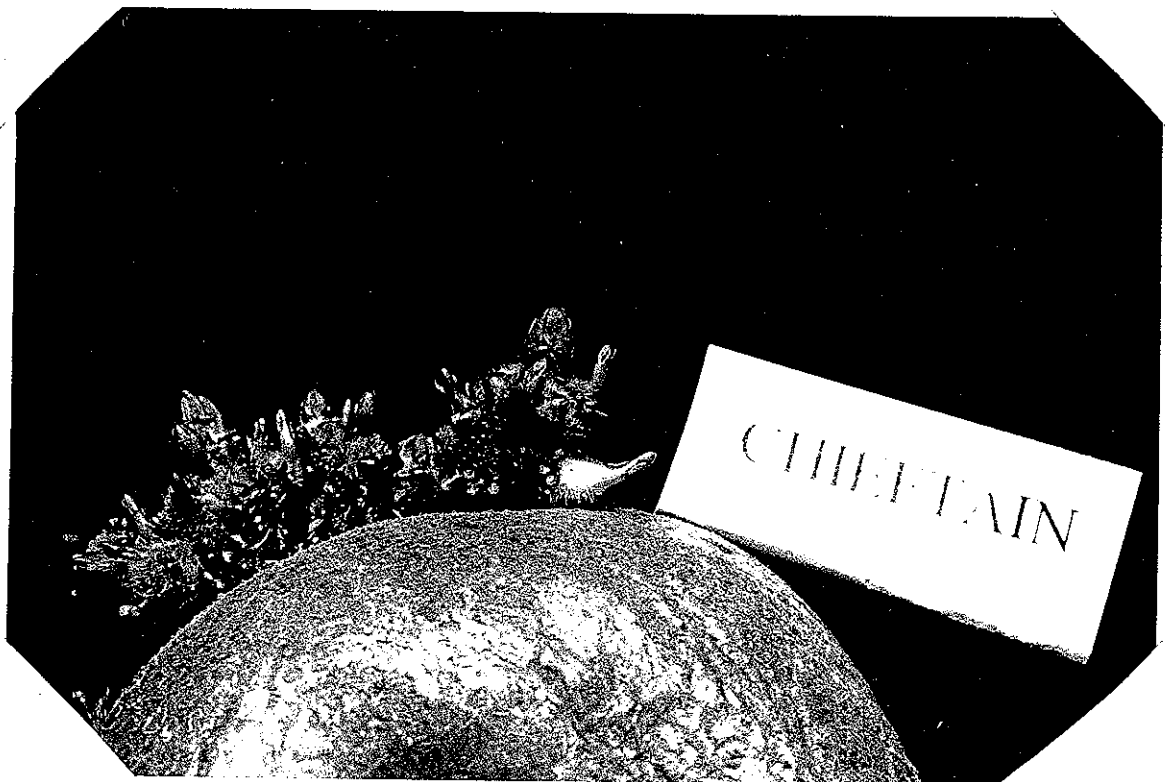
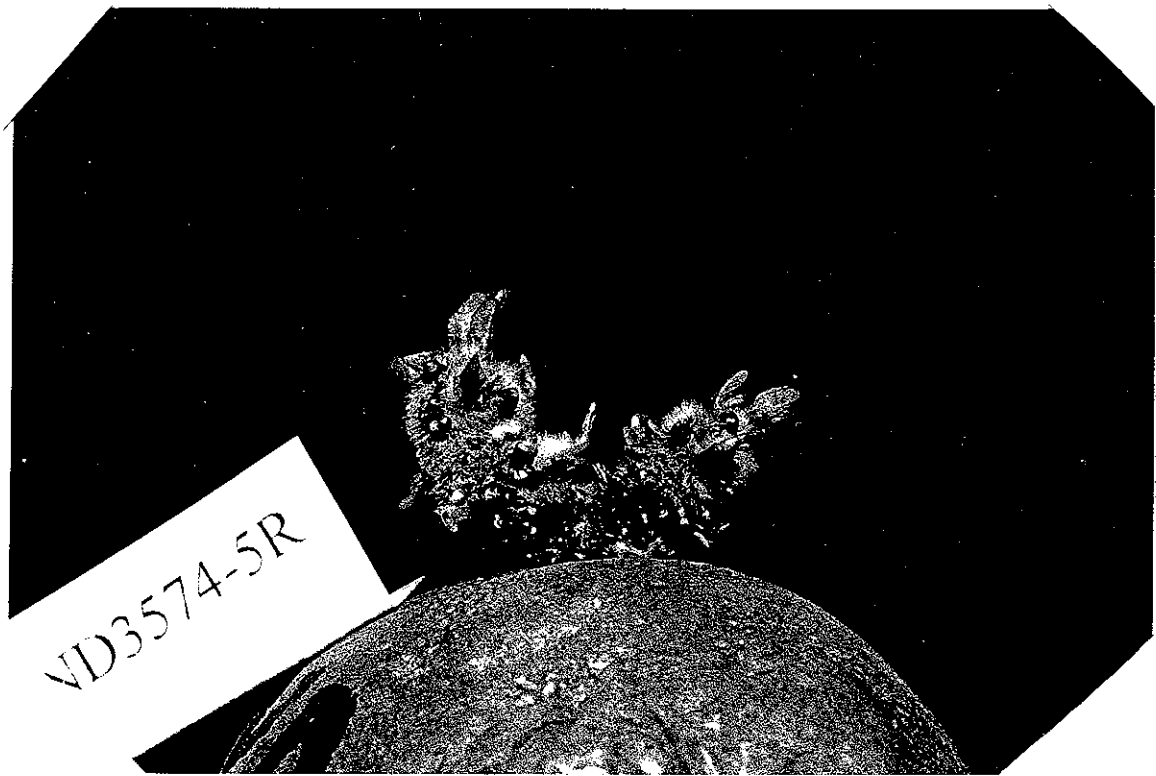
#### Literature Cited

Thompson, A.L., G.A. Secor, J.H. Lorenzen, B.L. Farnsworth, R.G. Novy, N.C. Gudmestad, E.T. Holm, and D.A. Preston. 2006. Dakota Rose: A bright red tablestock potato cultivar that retains its skin color in storage. Amer J Potato Res 83:317-323.

TABLE 1. Comparative traits for Dakota Rose, relative to Chieftain.

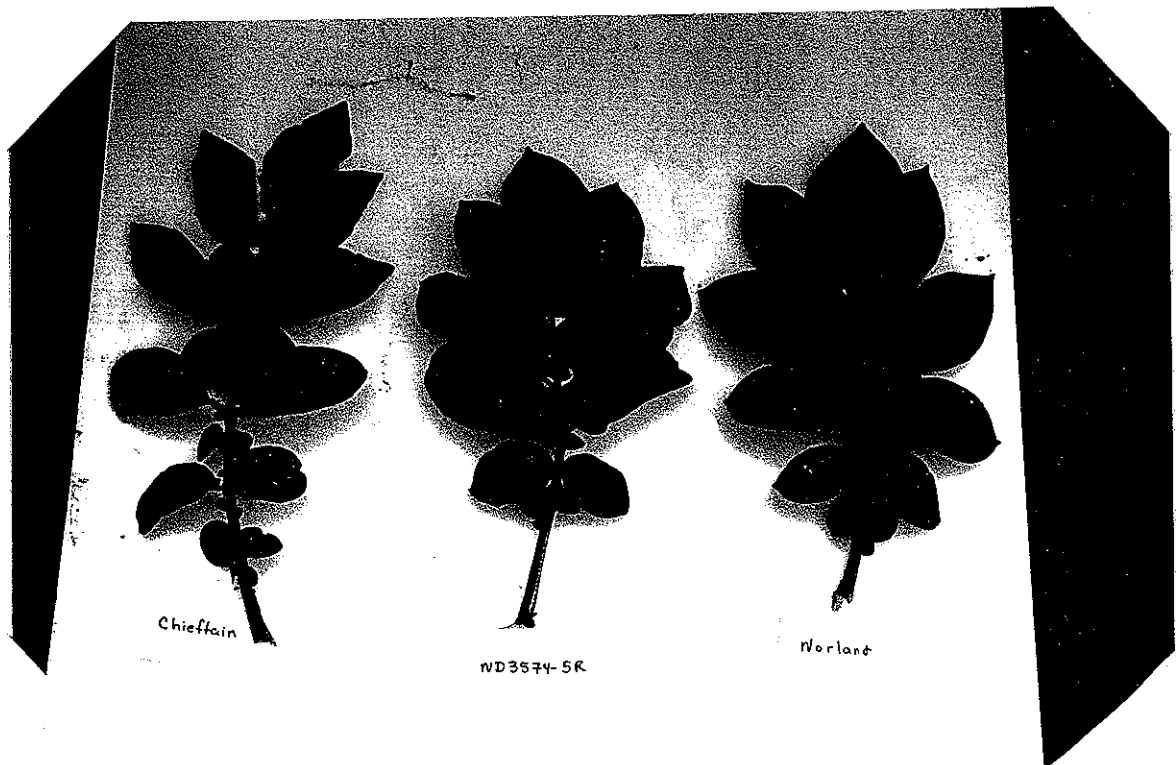
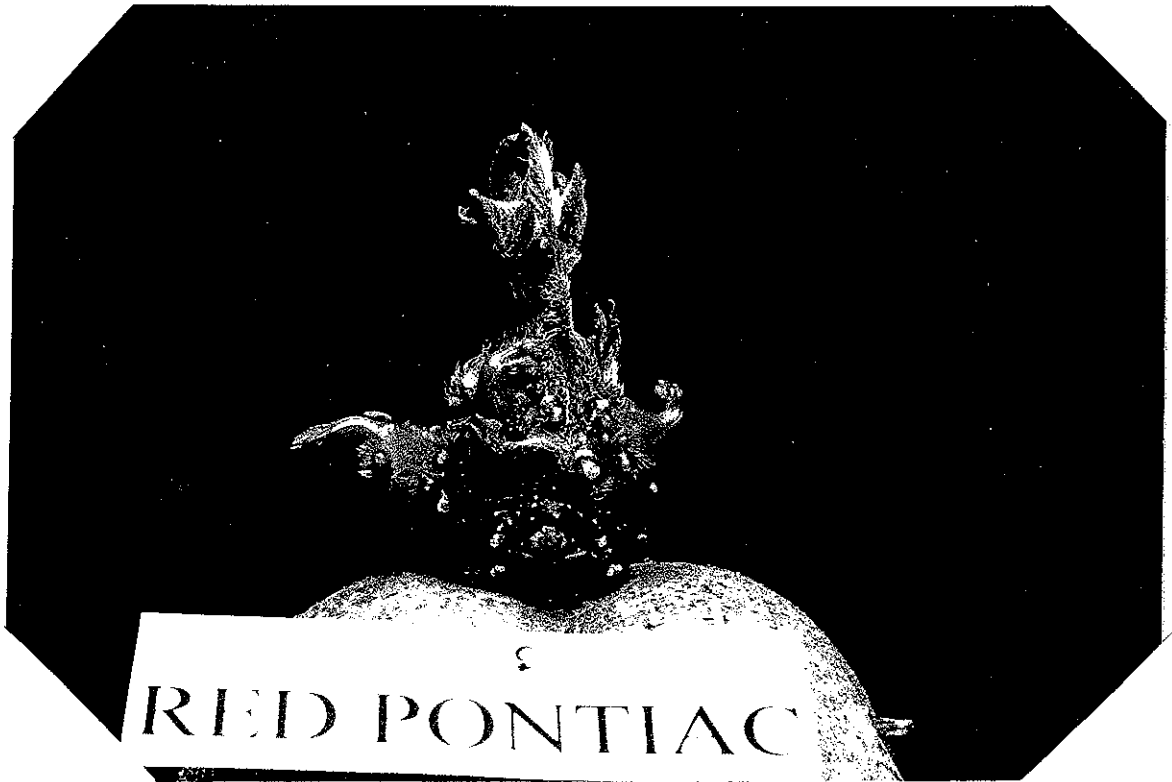
	Dakota Rose	Chieftain	Location of Evidence
1. Qualitative traits:			
Maturity	Early	Mid-season	Rating of 1-5 based on days after planting
Eyebrow prominence	Slight prominence	Medium prominence	Rating of 1-5, not prominent to Other
2. Color traits:			
Leaf color	Medium green	Medium green	146A (RHSCC)
Corolla (inner)	Dark red-purple	Pale red-purple	83C versus 85A (RHSCC)
Petiole Anthocyanin	Medium to strong	Medium	Rating 1-9, absent to very strong
Anther color	Bright yellow	Pale yellow	17B versus 14B (RHSCC)
3. Quantitative traits:			
Number 2° and 3° leaflet pairs	5.6	11	
Number of florets/inflorescence	14.4	10.2	
4. Other:			
Light sprout shape	Broad cylindrical	Broad cylindrical	Photograph
Light sprout base pubescence	Very strong	Strong	Photograph
Light sprout tip pubescence	Medium	Strong	Photograph
Light sprout tip anthocyanin intensity	Strong	Weak	Photograph

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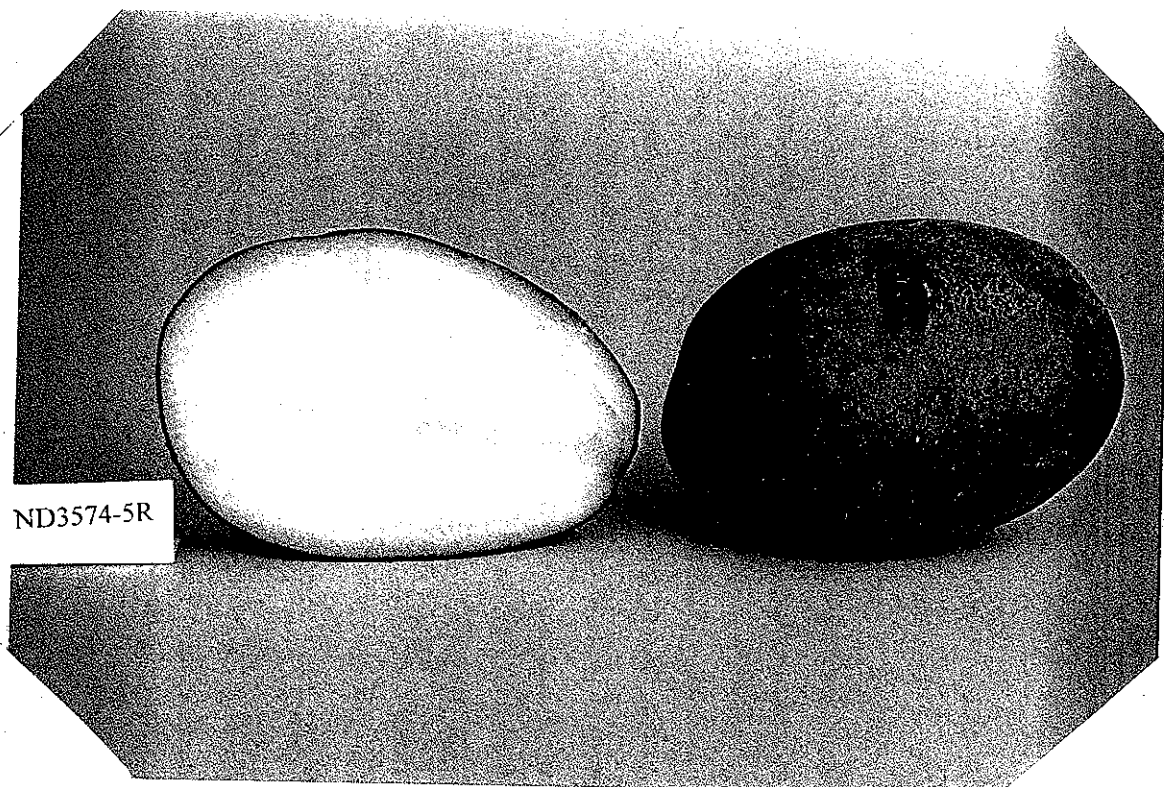
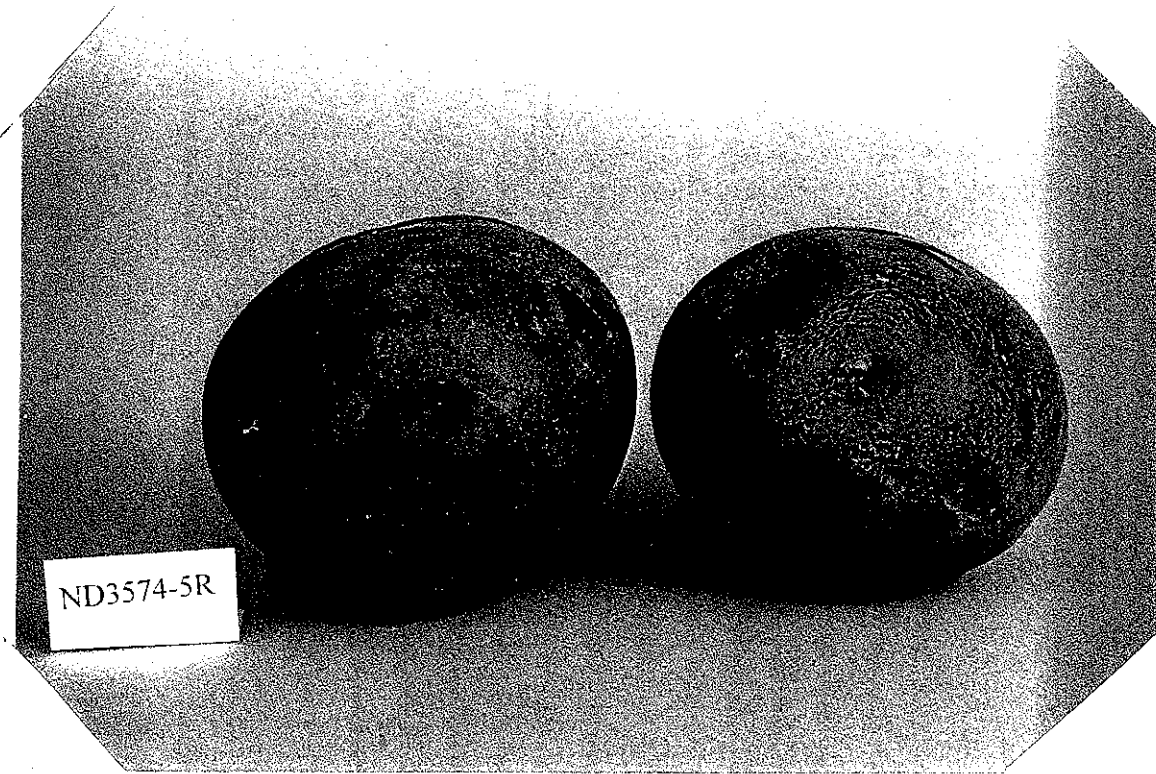
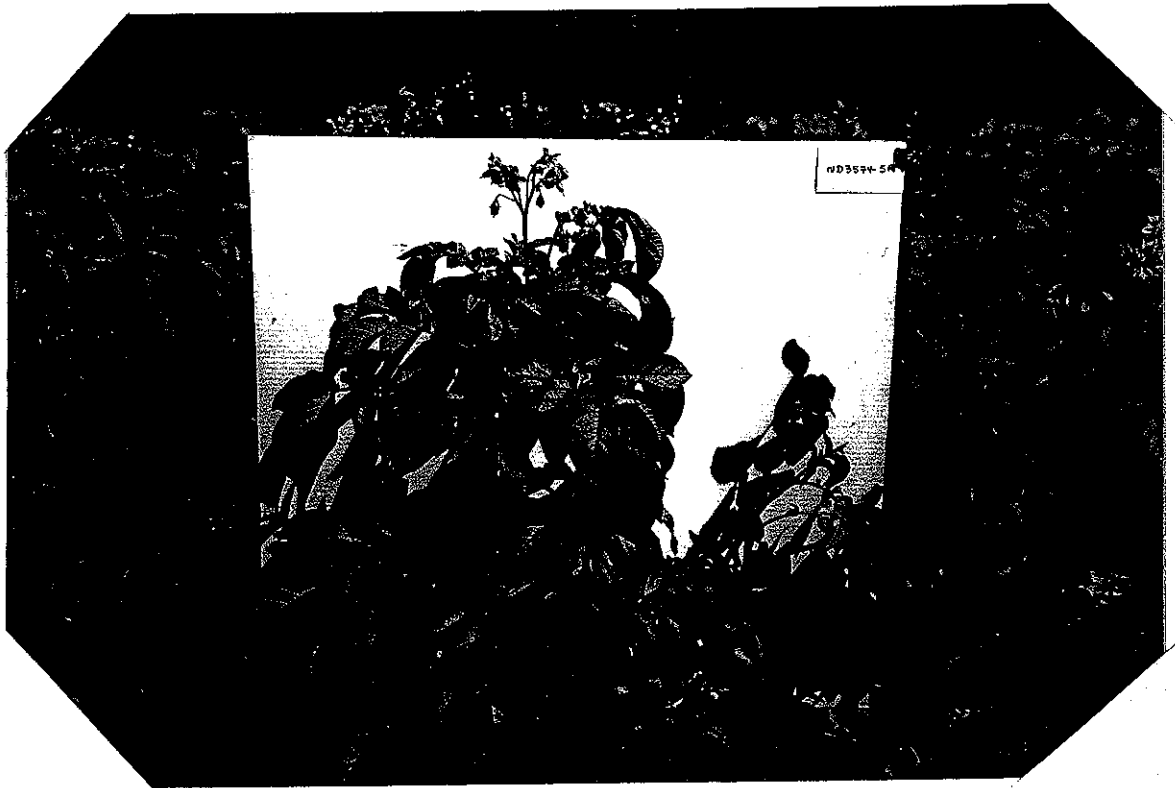


TABLE 1. Sensory evaluation of Dakota Rose, Red Norland, and Red Pontiac from 1993 to 2000.<sup>1</sup>

Baking												
Cultivar	Sloughing	Color	Color 4 h	Mealiness	Flavor	Baking			Microwaving			Summation of Values
						Mealiness	Flavor	Color	Mealiness	Flavor	Color	
1993-94												
Dakota Rose	10.0	10.0	8.5	5.4	8.0	9.5	5.2	9.5	10.0	5.0	7.3	88.4
Red Norland	10.0	9.3	8.5	6.3	7.7	9.0	6.0	9.0	9.0	5.6	7.8	88.2
Red Pontiac	9.8	9.3	8.8	5.6	7.7	8.0	6.0	8.0	8.5	6.8	7.9	86.4
1994-95												
Dakota Rose	-	7.5	6.0	3.4	6.5	8.0	3.7	8.0	7.0	2.9	5.9	58.9
Red Norland	-	6.5	5.5	4.3	6.0	7.5	5.3	7.5	7.0	5.4	7.4	62.4
Red Pontiac	-	8.0	8.0	6.0	7.4	7.0	4.5	7.0	7.0	3.7	6.0	64.6
1995-96												
Dakota Rose	8.0	7.5	6.5	3.7	6.0	8.0	4.9	8.0	8.5	4.7	6.1	71.9
Red Norland	4.5	7.0	5.5	4.4	6.3	7.8	4.5	7.8	7.8	4.9	6.4	66.9
Red Pontiac	5.5	7.0	6.5	5.0	6.2	7.5	5.6	7.5	7.5	5.1	6.1	69.5
1996-97												
Dakota Rose	8.3	8.8	6.5	2.9	4.4	8.3	3.6	8.3	8.5	3.7	5.0	68.3
Red Norland	5.3	7.0	7.3	4.6	5.9	8.0	4.3	8.0	7.3	4.2	6.0	67.9
Red Pontiac	5.5	7.5	8.5	5.1	6.3	7.0	4.9	7.0	7.5	4.6	6.0	69.9
1997-98												
Dakota Rose	9.0	9.3	7.3	2.5	4.0	9.5	3.3	9.5	8.0	3.3	4.7	70.4
Red Norland	9.0	9.0	7.5	3.4	5.5	9.0	4.9	9.0	9.3	4.7	5.8	77.1
Red Pontiac	8.5	9.5	8.7	4.6	6.0	9.0	4.4	9.0	9.3	4.2	6.0	79.2
1998-99												



Dakota Rose	8.7	9.2	8.3	3.4	4.9	9.2	3.6	9.2	9.0	3.8	5.3	74.6
Red Norland	9.2	8.7	8.3	4.6	4.7	8.8	4.8	8.8	8.8	4.6	5.9	77.2
Red Pontiac	8.3	9.7	9.0	4.9	6.0	8.8	4.9	8.8	9.0	4.1	5.1	78.6
1999-2000												
Dakota Rose	9.0	9.4	8.0	3.9	4.5	9.5	3.8	9.5	9.1	3.7	5.3	75.7
Red Norland	8.7	9.0	7.5	3.7	5.6	9.0	3.8	9.0	9.0	3.7	5.6	74.6
Red Pontiac	8.8	8.5	8.0	5.2	5.3	8.9	4.0	8.9	8.4	4.2	5.3	75.5
Overall Mean												
Dakota Rose	8.8	8.8	7.6	3.6	5.5	8.9	4.0	8.9	8.6	3.9	5.7	74.3
Red Norland	7.8	8.1	7.2	4.5	6.0	8.4	5.5	8.4	8.3	4.7	6.4	75.3
Red Pontiac	7.7	7.1	8.2	5.2	6.4	8.0	4.9	8.0	8.2	4.7	6.1	74.5

<sup>1</sup> Replicated samples of each entry were evaluated by a 3-5 member panel in a blind taste test. Characteristics are rated on a 1-9 scale, poor to excellent. Higher values are desirable.

## Dakota Rose: A Bright Red Tablestock Potato Cultivar That Retains Its Skin Color in Storage

A. L. Thompson<sup>\*1</sup>, G. A. Secor<sup>2</sup>, J. H. Lorenzen<sup>3</sup>, B. L. Farnsworth<sup>1</sup>, R. G. Novy<sup>4</sup>, N. C. Gudmestad<sup>2</sup>, E. T. Holm<sup>5</sup>, and D. A. Preston<sup>6</sup>

<sup>1</sup>Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA

<sup>2</sup>Department of Plant Pathology, North Dakota State University, Fargo, ND 58105, USA

<sup>3</sup>Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844, USA

<sup>4</sup>USDA-ARS, University of Idaho Research and Extension Center, Aberdeen, ID 83210, USA

<sup>5</sup>Department of Food and Nutrition, North Dakota State University, Fargo, ND 58105, USA

<sup>6</sup>University of Minnesota/North Dakota State University Extension, Old Business Hwy. #2, P.O. Box 301, East Grand Forks, MN 56721, USA

\*Corresponding author: Tel: 701-231-8160; Fax: 701-231-8174; Email: asunta.thompson@ndsu.edu

### ABSTRACT

'Dakota Rose' is a medium-maturing, white-fleshed, red-skinned cultivar that retains its bright red color in storage. Tubers have very smooth skin with an oblong shape. Yields are equivalent, or superior, to 'Red Norland', but lower than 'Red Pontiac', a late-maturing cultivar. Dakota Rose produces a high percentage of U.S. No. 1 tubers and few oversized tubers. Sensory evaluation scores for baked, boiled, and microwaved potatoes are similar to Red Norland and Red Pontiac, standard red tablestock cultivars. The specific gravity averaged about 1.067 across irrigated and non-irrigated sites, a typical value for a red tablestock cultivar. Adequate skin set for handling has often been difficult to achieve; application of nitrogen early in the growing season, coupled with chemical vinekill 3 weeks prior to harvest aids in minimizing the problem. Dakota Rose was released by the North Dakota Agricultural Experiment Station on 17 November 2000.

### RESUMEN

Dakota Rose es un cultivar de maduración intermedia, pulpa blanca, piel roja y que retiene su color rojo brillante después de almacenado. Los tubérculos tienen la piel muy lisa y forma oblonga. Los rendimientos son

equivalentes o superiores a Red Norland, pero menores que Red Pontiac, un cultivar de maduración tardía. Dakota Rose produce un alto porcentaje de tubérculos U.S. No 1 y unos pocos tubérculos de gran tamaño. La evaluación sensorial lo señala similar a Red Norland y Red Pontiac y a los cultivares rojos de mesa, papa asada, sancochada y en microondas. La gravedad específica promedió alrededor de 1.067 en lugares irrigados y no irrigados, un valor típico para un cultivar rojo para mesa. A menudo ha sido difícil conseguir la firmeza de la piel; la aplicación de nitrógeno a inicios de la época de cultivo, emparejado con la destrucción del follaje con un producto químico tres semanas antes de la cosecha, ayuda a minimizar el problema. Dakota Rose ha sido liberado por la Estación Experimental Agrícola de North Dakota, el 17 de noviembre del 2000.

### INTRODUCTION

'Dakota Rose' was evaluated as ND3574-5R and was released by the North Dakota Agricultural Experiment Station. Dakota Rose was derived from a cross originally made at NDSU on 22 March 1987 between North Dakota selections ND1196-2R and 'NorDonna'. The cultivars NorDonna, 'Norchief', 'Bison', and 'Reddale' are in the pedigree of Dakota Rose (Johansen et al. 1969, 1977; F. Lauer, unpublished; Novy et al. 1997). NorDonna is a dark red-skinned cultivar with wide adaptation that exhibits few internal defects (Novy et al. 1997). Attributes of Bison include the bright red skin color and very smooth tuber conformation. Yields of Bison are excellent

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ADDITIONAL KEY WORDS: *Solanum tuberosum*, cultivar, release, sensory evaluation

despite the small vine and maturity is medium, between 'Norland' (early) and 'Red Pontiac' (late) (Johansen et al. 1977). At its time of release, Norchief provided an alternative cultivar choice with better red skin color, over commercially available cultivars such as Norland and Red Pontiac (Johansen et al. 1957, 1969). Reddale, a 1984 release from Minnesota (F. Lauer, unpublished), was notable for its resistance to *Verticillium* wilt (*Verticillium albo-atrum* and *Verticillium dahliae*); however, commercial production was minimal due to the pale-red color of its skin. Dakota Rose combines many of the attributes of these cultivars including wide adaptability, bright red skin color, smooth tuber type, excellent yields, and relatively few defects have been noted during evaluation. The pedigree for Dakota Rose is presented in Figure 1.

Selection and early testing was done under the direction of Dr. Robert H. Johansen. The clone was initially selected at the Langdon Experiment Station at Langdon, ND, in 1988. Early evaluations were conducted at two locations in North Dakota. Advanced testing, seed increase, and commercial evaluation were done by several departments at NDSU and by certified seed and commercial producers in North Dakota and Minnesota. Breeders' seed was produced at the Horticultural Research Farm, Absaraka, ND, and the Agronomy Seed Farm, Casselton, ND. Subsequent increases were made by the North Dakota State Seed Department and cooperative certified seed producers under the guidance of the NDSU potato-breeding program and the NDSU Development Foundation. Dakota

Rose was widely evaluated in replicated trials at 11 locations in 8 years, and in regional trials at 10 North American sites (North Central states and Canadian provinces) in 1999 and 2000.

The name Dakota Rose was selected for the beautiful red skin color of this genotype. Dakota Rose is intended as a substitute or replacement for Red Pontiac and perhaps some acreage of 'Red LaSoda' and Red Norland. The color of Dakota Rose is superior to the color of all, and the tuber type is much smoother than both Red Pontiac and Red LaSoda. Additionally, it has significantly fewer internal disorders compared to Red Pontiac. Since its release, Dakota Rose has gained commercial acceptance primarily in Minnesota. In 2001, 10% of Minnesota's certified seed hectareage of red cultivars and 1% of North Dakota's, were planted to Dakota Rose (Certification Section of the Potato Association of America 2001). Dakota Rose accounted for 13.9% and 1.2% of hectareage of red cultivars certified in 2002, in Minnesota and North Dakota, respectively (Certification Section of the Potato Association of America 2002). In 2003, 14.0% of red cultivar hectareage certified in Minnesota and 3.8% in North Dakota were planted to Dakota Rose (Certification Section of the Potato Association of America 2003). During this time, U.S. certified seed acreage of Dakota Rose increased from 58.5 to 96.6 ha (Certification Section of the Potato Association of America 2001, 2002, 2003).

## DESCRIPTION

### Plants

*Growth habit:* large, vigorous, semi-erect vine, intermediate type; medium maturity. *Stems:* strong anthocyanin pigmentation; medium stem wings. *Leaves:* medium green (146A, Royal Horticultural Society Color Chart [RHSCC]); pubescence medium and short; leaf silhouette is medium; petiole anthocyanin pigmentation is medium to strong. *Terminal leaflets:* medium ovate shape with acute tip and obtuse to cordate base, weak leaflet margin waviness. *Primary leaflets:* medium sized, average of five pairs per leaf with narrowly ovate shape, acute tips, obtuse to cordate base shape. *Secondary and tertiary leaflets:* average of 5.6 pairs. *Stipules:* medium sized.

### Flowers

Peduncle consists of 15 to 25 buds and branches into two sections. The pedicel articulation is located two-thirds along

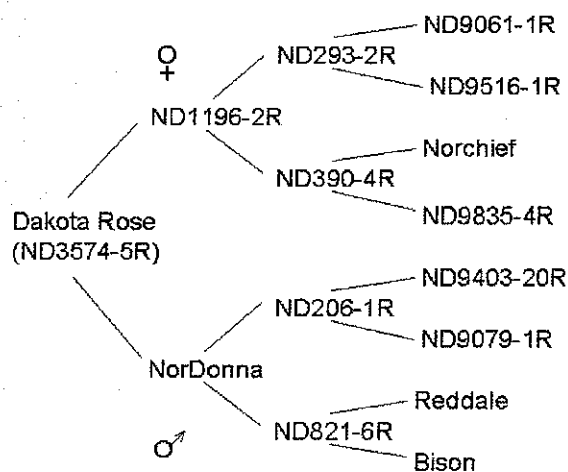
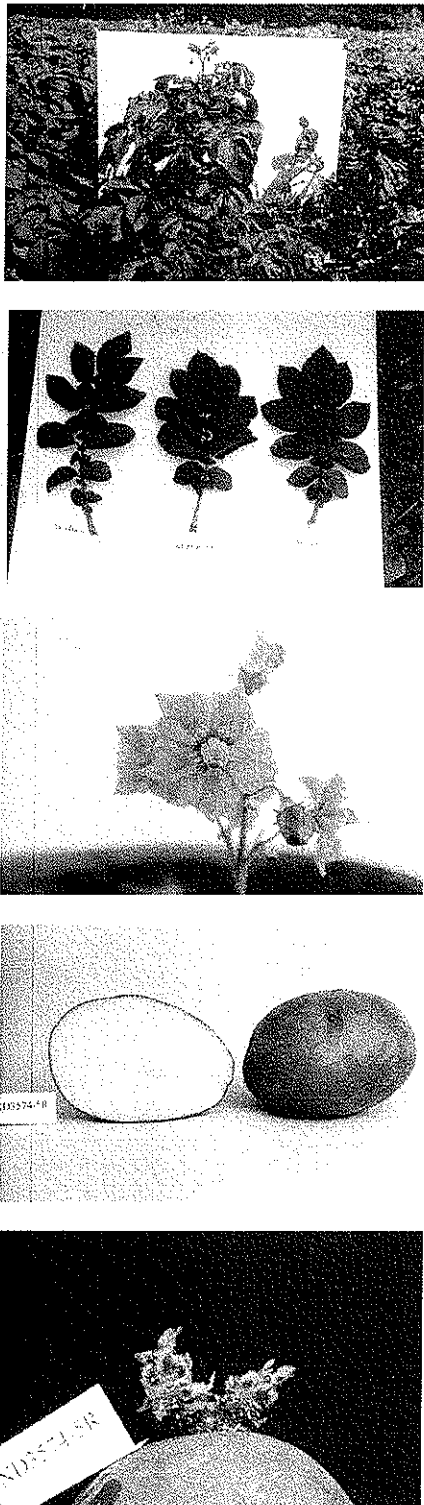


FIGURE 1.  
Pedigree of Dakota Rose.



**FIGURE 2.**  
Plant, leaf, flower, tuber, and sprout of Dakota Rose (ND3574-5R).

the length of the pedicel and is purple in color. *Buds*: heavily pubescent. *Calyx*: pubescent. *Corolla*: red-purple (83C, RHSCC), pentagonal in shape, with a mean diameter of 31 mm. *Anthers*: five; broad, cone shaped; golden yellow (17B RHSCC) with red pigmented tips; average length is 8 mm. *Pollen*: some; successful hybridizations occur with Dakota Rose used either as the female or male parent. *Stigma*: capitate; yellow-green (146B, RHSCC). *Berries*: production is low under field conditions.

### ***Tubers***

Tuber set is typically about eight tubers per plant. *Shape*: round to oblong and uniform; tubers grown under dryland conditions in North Dakota had a mean length of 8.23 cm  $\pm$  1.24, range 5.3 to 10.6 cm; mean width 6.02 cm  $\pm$  0.76, range 4.1 to 7.6 cm; mean thickness 5.30 cm  $\pm$  0.72, range 3.7 to 8.5 (measured from tubers 51 to 307 g, mean 149.8 g  $\pm$  56.4). *Skin*: bright red (60B, RHSCC) and smooth textured. *Eyes*: shallow, number about 10.6 per tuber, and are predominately apically distributed; eyebrows are slightly prominent. *Flesh*: white (158C, RHSCC). *Dormancy*: short.

### ***Light Sprouts***

Strong red-purple anthocyanin pigmentation at the base; broad cylindrical shape; medium sprout tip habit with medium pubescence, and strong red-purple sprout tip pigmentation; open and strongly hirsute bud and scales; rapid development; medium root initial frequency; lenticels not visible due to pubescence; few lateral shoots.

Morphological characteristics, including leaf, flower, tuber and light sprouts, are presented in Figure 2.

## **CHARACTERISTICS**

### ***Agronomic Performance***

Dakota Rose has been evaluated under irrigated and non-irrigated conditions in North Dakota, as well as in the North Central Regional Potato Variety Trials (NCRPVT). In non-irrigated trials conducted from 1993 to 2000, Dakota Rose averaged 20.1 mt/ha total yield, compared to 17.7 and 24.3 mt/ha, for Red Norland and Red Pontiac, respectively (Table 1). Dakota Rose averaged 82% U.S. No. 1 yield across locations and years; comparatively, Red Norland and Red Pontiac means were 74% and 83%, respectively. At non-irrigated sites, the specific gravity of Dakota Rose (1.069) was comparable to



TABLE 1—Total and U.S. No. 1 tuber yield and tuber specific gravity of Dakota Rose, Red Norland, and Red Pontiac potatoes grown in dryland trials at Grand Forks, Park River, and Hoople, ND, and Crookston, MN, from 1993 through 2000<sup>1</sup>.

Cultivar	Year	Tuber Yield			Specific Gravity
		Total	U.S. No. 1 <sup>2</sup>		
		mt/ha	%		
Grand Forks, Park River	1993				
Dakota Rose		24.6	21.4	87	1.071
Red Norland		16.7	13.0	78	1.076
Red Pontiac		21.5	16.4	76	1.076
Park River	1994				
Dakota Rose		27.0	21.1	78	1.066
Red Norland		24.6	17.0	69	1.072
Red Pontiac		23.7	19.7	83	1.070
Grand Forks	1995				
Dakota Rose		7.8	6.7	86	1.067
Red Norland		8.2	6.2	75	1.067
Red Pontiac		12.0	10.6	89	1.072
Grand Forks	1996				
Dakota Rose		7.3	4.1	57	1.068
Red Norland		4.0	1.8	44	1.072
Red Pontiac		10.1	7.6	76	1.073
Grand Forks, Park River	1997				
Dakota Rose		18.9	13.4	70	1.068
Red Norland		15.7	8.4	59	1.072
Red Pontiac		24.2	20.4	80	1.070
Crookston	1998				
Dakota Rose		31.0	28.8	92	-
Red Norland		23.7	21.6	91	1.073
Red Pontiac		32.5	27.0	83	1.078
Hoople	1999				
Dakota Rose		21.5	20.2	94	1.072
Red Norland		29.4	25.8	88	1.074
Red Pontiac		44.2	40.5	92	1.079
Crookston, Hoople	2000				
Dakota Rose		22.5	19.3	90	1.070
Red Norland		19.5	17.6	85	1.070
Red Pontiac		25.8	22.7	88	1.071
Overall Mean					
Dakota Rose		20.1	16.9	82	1.069
Red Norland		17.7	13.9	74	1.072
Red Pontiac		24.3	20.6	83	1.074

<sup>1</sup>Flooding of research plot sites compromised yields in 1994, 1995, 1996, and 1997 at Grand Forks. The Hoople site was very dry early in 2000, resulting in lower yields.

<sup>2</sup>U.S. No. 1 tubers reported as  $\geq 5.08$  cm diameter.

that of the standard cultivars, Red Norland (1.072) and Red Pontiac (1.074).

Total yields of Dakota Rose at irrigated sites in North Dakota were 33.4 mt/ha averaged across years and locations (Table 2). Red Norland and Red Pontiac averaged 36.2 and 44.9 mt/ha, respectively, in the same trials. Overall means for percentage of U.S. No. 1 yields were excellent for Dakota Rose, Red Norland, and Red Pontiac, with values of 92%, 88%, and 90%, respectively. The specific gravity of all cultivars was lower under irrigated production than under non-irrigated culture.

Performance in the NCRPVT indicates Dakota Rose is widely adapted. The average total and U.S. No. 1 yield across 10 North American sites during 1999 and 2000 was 39.0 and 32.1 mt/ha, compared to 35.8 and 29.4 mt/ha for Dark Red Norland, and 49.2 and 39.9 for Red Pontiac (Table 3). Specific gravity for Dakota Rose in the NCRPVT was slightly lower compared to the standard tablestock cultivars. Cooperators ranked Dakota Rose as superior to Dark Red Norland and Red Pontiac in each of the two years.

### Biochemical and Culinary Quality Characteristics

Total glycoalkaloid levels for Dakota Rose are low (Table 4). Dakota Rose averaged 3.37 mg/100 g fresh potato tuber tissue compared to check cultivars Lenape and Russet Burbank at 18.84 and 7.18, respectively.

Dakota Rose produces attractive bright-red tubers that have consumer appeal. The mean summation of sensory evaluation for baked, boiled, and microwaved products for Dakota Rose was 69.1, compared to 71.1 and 72.8 for Red Norland and Red Pontiac (Table 5). Mealiness scores were lower for Dakota Rose, indicating a more waxy texture and making this cultivar particularly suited for use in potato salads and soups; specific gravity levels confirm this as well (Tables 1 and 2). In trials, hollow heart was minimal for Dakota Rose and Red Norland, but was noted occasionally in Red Pontiac.

### Isozyme and DNA Fingerprinting Profiles

Isoelectric focusing (IEF) electrophoresis was performed on extracts of potato tuber protein using the method and gels developed by Perkin-Elmer Life Sciences, Norton, OH. Separate tests were conducted for tuber proteins and the enzyme peroxidase on the following samples: Dakota Rose, NorDonna, Red Norland, Red Pontiac, and 'Chipeta'.

IEF analysis of tuber proteins stained with a general protein stain (brilliant blue R-250) revealed unique protein banding patterns or fingerprints for these cultivars. The diagnostic area of the gel was found in the 40- to 70-mm range as measured from the edge of the anode wick towards the cathode wick. Dakota Rose had prominent bands at 42, 56, 59, 60, 63, 66, and 68 mm. NorDonna has major bands at 42, 56, 59, 60, 62, 66, and 68 mm. The band at 62 mm was not found in Dakota Rose. The protein-banding pattern for Red Norland resulted in major bands at 41, 42, 56, 59, 60, 63, 66, and 68 mm. The fingerprint for Red Pontiac resulted in nine prominent bands at 40.5, 41, 42, 56, 58.5, 62, 63, 66, and 68 mm. IEF analysis of the enzyme peroxidase also revealed unique banding patterns for these five cultivars.

### Disease Response, Bruising, and Physiological Disorders

During evaluation, Dakota Rose exhibited no notable disease or pest resistance, nor exceptional susceptibility. Symptom expression of bacterial ring rot (*Clavibacter michiganensis* subsp. *sepe-donicus*) is typical for both vine and tubers. Foliar expression, including marginal leaf necrosis, leaf rolling, and interveinal chlorosis, was first observed 80 to 90 days after planting in the Red River Valley. Tuber symptoms include periderm cracking, vascular breakdown, and cheesy exudate. Dakota Rose exhibits typical foliar symptom expression when infected by *Potato virus Y* (PVY<sup>a</sup>). Dakota Rose possesses some resistance to common scab (*Streptomyces scabies*) and silver scurf (*Helminthosporium solani*). Incidence of hollow heart is very low.

TABLE 2—Total and U.S. No. 1 tuber yield and tuber specific gravity of Dakota Rose, Red Norland, and Red Pontiac potatoes grown in irrigated trials at Carrington, Dawson, Oakes, Larimore, McCanna, and McLeod, ND, and Glyndon, MN, from 1993 to 2000<sup>1</sup>.

Cultivar	Year	Tuber Yield		Specific Gravity	
		Total	U.S. No. 1 <sup>2</sup>		
			mt/ha		%
Oakes	1993				
Dakota Rose		11.0	8.6	79	1.063
Red Norland		22.3	20.0	90	1.073
Red Pontiac		26.1	23.4	90	1.070
Carrington, Dawson, Oakes	1994				
Dakota Rose		37.4	35.2	94	1.063
Red Norland		45.7	42.7	93	1.072
Red Pontiac		48.0	45.2	94	1.069
	1995				
Dakota Rose		43.0	40.2	94	1.068
Red Norland		40.2	37.1	92	1.067
Red Pontiac		41.8	39.9	95	1.066
	1996				
Dakota Rose		43.3	41.7	96	1.065
Red Norland		43.6	38.9	89	1.072
Red Pontiac		56.3	52.4	93	1.065
McCanna, Oakes	1997				
Dakota Rose		44.2	40.6	92	1.059
Red Norland		39.6	35.2	87	1.061
Red Pontiac		52.2	46.2	88	1.067
McCanna, McLeod	1998				
Dakota Rose		29.3	27.3	88	1.061
Red Norland		26.5	22.8	86	1.061
Red Pontiac		47.0	42.7	91	1.063
McCanna, Glyndon	1999				
Dakota Rose		27.2	23.5	86	1.057
Red Norland		30.0	23.7	77	1.063
Red Pontiac		45.0	38.8	86	1.063
Dawson, Larimore	2000				
Dakota Rose		31.7	27.7	88	1.078
Red Norland		41.7	36.7	88	1.083
Red Pontiac		43.0	36.6	86	1.068
Overall Mean					
Dakota Rose		33.4	30.6	92	1.064
Red Norland		36.2	32.1	88	1.069
Red Pontiac		44.9	40.7	90	1.066

<sup>1</sup>The yields in the McLeod trial were low due to flooding in 1998. Glyndon 1999 trial yields were compromised due to hail. Larimore received excessive rains in 2000.

<sup>2</sup>U.S. No. 1 tubers reported as  $\geq 5.08$  cm diameter.

TABLE 3—Total and U.S. No. 1 tuber yield, specific gravity, and ranking of *Dakota Rose*, *Dark Red Norland*, and *Red Pontiac* potatoes grown in North Central Regional Potato Variety Trials in 1999 and 2000<sup>1</sup>.

Cultivar	Year	Tuber Yield		Specific Gravity	Rate <sup>2</sup>
		Total	U.S. No. 1 <sup>2</sup>		
		mt/ha	%		
	1999				
Dakota Rose		36.0	29.8	1.063	5
Dark Red Norland		33.3	27.2	1.065	1
Red Pontiac		46.8	38.1	1.066	2
	2000				
Dakota Rose		41.9	34.3	1.065	14
Dark Red Norland		38.2	31.5	1.069	10
Red Pontiac		51.5	41.6	1.066	10
Overall Mean					
Dakota Rose		39.0	32.1	1.064	10
Dark Red Norland		35.8	29.4	1.067	6
Red Pontiac		49.2	39.9	1.066	6

<sup>1</sup>NCRPVT was grown at 10 locations in 1999 and 2000, including seven states and three Canadian provinces. Reported means are for dryland and irrigated sites combined.

<sup>2</sup>U.S. No. 1 yield reported as  $\geq 5.08$  cm diameter.

<sup>3</sup>Ranking determined by merit rating values of 1 to 5 with point values corresponding to 5 to 1. Value reported equals total points earned; the higher the value, the greater the merit.

TABLE 4—Glycoalkaloid<sup>1</sup> levels for *Dakota Rose* tubers relative to check genotypes, grown at Absaraka and Wyndmere, ND, in 2004.

Clone	Solanine	Chaconine	Total Glycoalkaloids
			mg/100 g fresh weight basis
Absaraka			
Dakota Rose	1.07	0.78	1.85
Lenape	7.75	12.55	20.30
Russet Burbank	3.77	3.42	7.18
Wyndmere			
Dakota Rose	2.82	2.06	4.88
Lenape	6.48	10.90	17.38
Russet Burbank	-	-	-

<sup>1</sup>Procedure based on Lafta and Lorenzen (2000), modified for tubers, which does not require heating during extraction of tuber glycoalkaloids.

## UTILIZATION

Consumer appeal of *Dakota Rose* as a tablestock cultivar is attributed to the bright red skin and attractive tuber appearance. Improved retention of skin color following storage, relative to other red-skinned cultivars, is also a desirable attribute of *Dakota Rose*. Sensory evaluation scores following baking, boiling, and microwaving indicate *Dakota Rose* has similar ratings to *Red Pontiac* and *Red Norland* (Table 5). Its waxy tex-

ture makes *Dakota Rose* well suited for use in soups, stews, and potato salads.

## MANAGEMENT

*Dakota Rose* seems best suited to dryland production, with excellent grower production resulting on peat soils. Results from yield trials, in addition to grower experience, in North Dakota, Minnesota, and North Central Regional Trials indicate that adequate skin set for handling is often difficult to achieve. Skinning is often noted with an associated occurrence of soft rot in storage. Suggested management techniques to minimize this problem include minimal N fertilization applied in a timely manner, early in the growing season, to encourage vine senescence, tuber maturity, and subsequent skin set. Additionally, chemical vine desiccation 21 days prior to

desired harvest date may encourage skin maturation. Because *Dakota Rose* has short dormancy, sprout inhibition and/or use of cold storage temperatures (3.3 C) are suggested for tablestock potatoes.

## AVAILABILITY

Plant Variety Protection was sought on behalf of the NDSU Research Foundation for *Dakota Rose*; certificate 200100227 is pending. Tissue-culture-based limited-generation seed production, including micro-propagated plantlets and minitubers, are available from the North Dakota State Seed Department and others. Certified seed of *Dakota Rose* is available from producers in North Dakota and Minnesota. Small amounts for research purposes may be obtained by contacting the corresponding author.

## ACKNOWLEDGMENTS

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TABLE 5—Sensory evaluation of Dakota Rose, Red Norland, and Red Pontiac from 1993 to 2000.<sup>1</sup>

Cultivar	Sloughing	Color	Boiling			Baking			Microwaving			Summation of Values
			Color	4 h	Mealiness	Flavor	Color	Mealiness	Flavor	Color	Mealiness	
1993-94												
Dakota Rose	10.0	10.0	8.5	5.4	8.0	9.5	5.2	9.5	10.0	5.0	7.3	88.4
Red Norland	10.0	9.3	8.5	6.3	7.7	9.0	6.0	9.0	9.0	5.6	7.8	88.2
Red Pontiac	9.8	9.3	8.8	5.6	7.7	8.0	6.0	8.0	8.5	6.8	7.9	86.4
1994-95												
Dakota Rose	-	7.5	6.0	3.4	6.5	8.0	3.7	8.0	7.0	2.9	5.9	58.9
Red Norland	-	6.5	5.5	4.3	6.0	7.5	5.3	7.5	7.0	5.4	7.4	62.4
Red Pontiac	-	8.0	8.0	6.0	7.4	7.0	4.5	7.0	7.0	3.7	6.0	64.6
1995-96												
Dakota Rose	8.0	7.5	6.5	3.7	6.0	8.0	4.9	8.0	8.5	4.7	6.1	71.9
Red Norland	4.5	7.0	5.5	4.4	6.3	7.8	4.5	7.8	7.8	4.9	6.4	66.9
Red Pontiac	5.5	7.0	6.5	5.0	6.2	7.5	5.6	7.5	7.5	5.1	6.1	69.5
1996-97												
Dakota Rose	8.3	8.8	6.5	2.9	4.4	8.3	3.6	8.3	8.5	3.7	5.0	68.3
Red Norland	5.3	7.0	7.3	4.6	5.9	8.0	4.3	8.0	7.3	4.2	6.0	67.9
Red Pontiac	5.5	7.5	8.5	5.1	6.3	7.0	4.9	7.0	7.5	4.6	6.0	69.9
1997-98												
Dakota Rose	9.0	9.3	7.3	2.5	4.0	9.5	3.3	9.5	8.0	3.3	4.7	70.4
Red Norland	9.0	9.0	7.5	3.4	5.5	9.0	4.9	9.0	9.3	4.7	5.8	77.1
Red Pontiac	8.5	9.5	8.7	4.6	6.0	9.0	4.4	9.0	9.3	4.2	6.0	79.2
1998-99												
Dakota Rose	8.7	9.2	8.3	3.4	4.9	9.2	3.6	9.2	9.0	3.8	5.3	74.6
Red Norland	9.2	8.7	8.3	4.6	4.7	8.8	4.8	8.8	8.8	4.6	5.9	77.2
Red Pontiac	8.3	9.7	9.0	4.9	6.0	8.8	4.9	8.8	9.0	4.1	5.1	78.6
1999-2000												
Dakota Rose	9.0	9.4	8.0	3.9	4.5	9.5	3.8	9.5	9.1	3.7	5.3	75.7
Red Norland	8.7	9.0	7.5	3.7	5.6	9.0	3.8	9.0	9.0	3.7	5.6	74.6
Red Pontiac	8.8	8.5	8.0	5.2	5.3	8.9	4.0	8.9	8.4	4.2	5.3	75.5
Overall Mean												
Dakota Rose	8.8	8.8	7.6	3.6	5.5	8.9	4.0	8.9	8.6	3.9	5.7	74.3
Red Norland	7.8	8.1	7.2	4.5	6.0	8.4	5.5	8.4	8.3	4.7	6.4	75.3
Red Pontiac	7.7	7.1	8.2	5.2	6.4	8.0	4.9	8.0	8.2	4.7	6.1	74.5

<sup>1</sup>Replicated samples of each entry were evaluated by a three- to five-member panel in a blind taste test. Characteristics are rated on a 1-9 scale, poor to excellent. Higher values are desirable.

Funding to support the development of Dakota Rose came from the North Dakota State Agricultural Experiment Station, USDA-ARS/CSREES, and the Red River Valley Potato Grower's Association.

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#200100227

Exhibit C

## **OBJECTIVE DESCRIPTION OF VARIETY**

**DAKOTA ROSE**

**Undine Road, NB  
2000**

*Global Agri Services Inc.*

**OBJECTIVE DESCRIPTION OF VARIETY**POTATO (*Solanum tuberosum* L.)

NAME OF APPLICANT(S)

NDSU Research Foundation

FOR OFFICIAL USE ONLY

PVPO NUMBER #200100227

ADDRESS

P.O. Box 5014

1735 NDSU Research Park Drive

Fargo, ND 58105-5002

VARIETY NAME

DAKOTA ROSE

TEMPORARY OR EXPERIMENTAL  
DESIGNATION

REFERENCE VARIETY 1 (R1)

REFERENCE VARIETY 2 (R2)

Chieftain

Red Pontiac

**1. MARKET CHARACTERISTICS:**

MARKET CLASS:

1 = Yellow-flesh tablestock; 2 = Round-white tablestock; 3 = Chip-processing; 4 = Frozen-processing

5 = Russet tablestock; 6 = Other

VARIETY	R1	R2
6 Red tablestock	6 Red tablestock	6 Red tablestock

**2. PLANT CHARACTERISTICS:**

GROWTH HABIT:

1 = Erect (&gt;45° with ground); 5 = Semi-erect (30-45° with ground); 7 = Spreading.

VARIETY	R1	R2
5	5	5

TYPE:

1 = Stem (foliage open, stems clearly visible); 2 = Intermediate; 3 = Leaf (foliage closed, stems hardly visible)

VARIETY	R1	R2
2	2-3	2

MATURITY: Days after planting (DAP) at vine senescence.

VARIETY	R1	R2

PLANTING DATE:

VARIETY	R1	R2
May 17, 2000	May 17, 2000	May, 1999

REGION/AREA:

VARIETY	R1	R2
Undine Rd., New Brunswick	Undine Rd., New Brunswick	North Tilley, New Brunswick

## MATURITY CLASS:

1 = Very Early (<100 DAP); 2 = Early (100-110 DAP); 3 = Mid-season (111-120 DAP); 4 = Late (121-130 DAP); 5 = Very Late (> 130 DAP).

VARIETY	R1	R2
2	3	4

## 3. STEM CHARACTERISTICS: Measure at early first bloom

## STEM ANTHOCYANIN COLORATION:

1 = Absent; 3 = Weak; 5 = Medium; 7 = Strong; 9 = Very Strong

VARIETY	R1	R2
7	5	5

## STEM WINGS

1 = Absent; 3 = Weak; 5 = Medium; 7 = Strong; 9 = Very Strong

VARIETY	R1	R2
5	7	7

## 4. LEAF CHARACTERISTICS:

LEAF COLOR: Observe fully developed leaves located on middle 1/3 of plant

1 = Yellowish-green; 2 = Olive-green; 3 = Medium green; 4 = Dark green; 5 = Grey-green; 6 = other

VARIETY	R1	R2
3	3	4

LEAF COLOR: Observe fully developed leaves located on middle 1/3 of plant

Royal Horticulture Society Color Chart

VARIETY	R1	R2
146A	146A	147A

## LEAF PUBESCENCE DENSITY:

1 = Absent; 2 = Sparse; 3 = Medium; 4 = Thick; 5 = Heavy

VARIETY	R1	R2
3	3	3

## LEAF PUBESCENCE LENGTH:

1 = None; 2 = Short; 3 = Medium; 4 = Long; 5 = Very long

VARIETY	R1	R2
2	2	2

## LEAF SILHOUETTE

1 = Closed; 3 = Medium; 5 = Open

VARIETY	R1	R2
3	3	3

## PETIOLES ANTHOCYANIN COLORATION:

1 = Absent; 3 = Weak; 5 = Medium; 7 = Strong; 9 = Very Strong

VARIETY	R1	R2
5-7	5	3

## LEAF STIPULES SIZE

1 = Absent; 3 = Small; 5 = Medium; 7 = Large

VARIETY	R1	R2
5	7	5

## TERMINAL LEAFLET SHAPE:

1 = Narrowly ovate; 2 = Medium ovate; 3 = Broadly ovate; 4 = Lanceolate; 5 = Elliptical;  
6 = Obovate; 7 = Oblong; 8 = other

VARIETY	R1	R2
2	2	2

## TERMINAL LEAFLET TIP SHAPE:

1 = Acute; 2 = Cuspidate; 3 = Acuminate; 4 = Obtuse; 5 = other

VARIETY	R1	R2
1	1	1

## TERMINAL LEAFLET BASE SHAPE:

1 = Cuneate; 2 = Acute; 3 = Obtuse; 4 = Cordate; 5 = Truncate; 6 = Lobed; 7 = Other

VARIETY	R1	R2
3-4	3-4	4

## TERMINAL LEAFLET MARGIN WAVINESS:

1 = Absent; 2 = Slight; 3 = Weak; 4 = Medium; 5 = Strong

VARIETY	R1	R2
3	3	3

## NUMBER OF PRIMARY LEAFLET PAIRS:

## AVERAGE:

VARIETY	R1	R2
5	6	5.1

## RANGE:

VARIETY	R1	R2
5	6	5-6

## PRIMARY LEAFLET TIP SHAPE:

1 = Acute; 2 = Cuspidate; 3 = Acuminate; 4 = Obtuse; 5 = Other

VARIETY	R1	R2
1	1	1



## PRIMARY LEAFLET SIZE

1 = Very small; 2 = small; 3 = Medium; 4 = Large; 5 = Very large

VARIETY	R1	R2
3	2-3	2-3

## PRIMARY LEAFLET SHAPE:

1 = Narrowly ovate; 2 = Medium ovate; 3 = Broadly ovate; 4 = Lanceolate; 5 = Elliptical;  
6 = Obovate; 7 = Oblong; 8 = Other

VARIETY	R1	R2
1	1	1

## PRIMARY LEAFLET BASE SHAPE:

1 = Cuneate; 2 = Acute; 3 = Obtuse; 4 = Cordate; 5 = Truncate; 6 = Lobed; 7 = Other

VARIETY	R1	R2
3-4	4	4

## NUMBER OF SECONDARY AND TERTIARY LEAFLET PAIRS:

## AVERAGE:

VARIETY	R1	R2
5.6	11	6.9

## RANGE:

VARIETY	R1	R2
4-7	8-12	6-9

## 5. INFLORESCENCE CHARACTERISTICS:

## NUMBER OF INFLORESCENCE / PLANT

## AVERAGE

VARIETY	R1	R2
3.6	3	2.4

## RANGE:

VARIETY	R1	R2
1-5	3	1-4

## NUMBER OF FLORETS / INFLORESCENCE:

## AVERAGE:

VARIETY	R1	R2
14.4	10.2	13.0

## RANGE:

VARIETY	R1	R2
7-17	7-13	6-18

## COROLLA INNER SURFACE COLOR: Measure predominant color of newly open flower RHSCC.

VARIETY	R1	R2
83C	85A	86C

## COROLLA OUTER SURFACE COLOR: RHSCC

VARIETY	R1	R2
83C	76A	86D

## COROLLA SHAPE:

1 = Very rotate; 2 = Rotate; 3 = Pentagonal; 4 = Semi-stellate; 5 = Stellate

VARIETY	R1	R2
3	3	3

## CALYX ANTHOCYANIN COLORATION:

1 = Absent; 3 = Weak; 5 = Medium; 7 = Strong; 9 = Very strong

VARIETY	R1	R2
7	2	3

## ANTHER COLOR: Measure when newly opened flower is fully expanded RHSCC

VARIETY	R1	R2
17B	14B	17B

## ANTHER SHAPE:

1 = Broad cone; 2 = Narrow cone; 3 = Pear shape cone; 4 = Loose; 5 = Other

VARIETY	R1	R2
1 dark red tips	2	3

## POLLEN PRODUCTION:

1 = None; 3 = Some; 5 = Abundant

VARIETY	R1	R2
3	—	—

## STIGMA SHAPE:

1 = Capitate; 2 = Clavate; 3 = Bi-lobed

VARIETY	R1	R2
1	1	1

## STIGMA COLOR: RHSCC

VARIETY	R1	R2
146B	148A	146A

## BERRY PRODUCTION: UNDER FIELD CONDITIONS:

1 = None; 3 = low; 5 = Moderate; 7 = Heavy; 9 = Very heavy

VARIETY	R1	R2
3	—	—

## 6. TUBER CHARACTERISTICS:

## PREDOMINANT SKIN COLOR:

1 = White; 2 = Light Yellow; 3 = Yellow; 4 = Buff; 5 = Tan; 6 = Brown; 7 = Pink; 8 = red;  
 9 = Purplish-red; 10 = Purple; 11 = Dark purple-black; 12 = Other

VARIETY	R1	R2
8	8	8

## RHSCC:

VARIETY	R1	R2
60B	60C	60D

## SECONDARY SKIN COLOR:

1 = Absent; 2 = Present, please describe

VARIETY	R1	R2
1	1	1

## RHSCC:

VARIETY	R1	R2
—	—	—

## SECONDARY SKIN COLOR DISTRIBUTION:

1 = Eyes; 2 = Eyebrows; 3 = Splashed; 4 = Scattered; 5 = Spectacled; 6 = Stippled; 7 = Other

VARIETY	R1	R2
—	—	—

## SKIN TEXTURE:

1 = Smooth; 2 = Rough (flaky); 3 = Netted; 4 = Russetted; 5 = Heavily russetted; 6 = Other

VARIETY	R1	R2
1	1	2

## TUBER SHAPE:

1 = Compressed; 2 = Round; 3 = Oval; 4 = Oblong; 5 = Long; 6 = Other

VARIETY	R1	R2
2-3	2-3	2

## TUBER THICKNESS:

1 = Round; 2 = Medium thick; 3 = Slightly flattened; 4 = Flattened; 5 = Other

VARIETY	R1	R2
2	3	1

## TUBER LENGTH (mm):

## AVERAGE:

VARIETY	R1	R2
72.3	76.2	69.3

## RANGE:

VARIETY	R1	R2
60-85	64-90	56-83

## STANDARD DEVIATION:

VARIETY	R1	R2
5.4	6.1	7.2

## AVERAGE WEIGHT OF SAMPLE TAKEN:

VARIETY	R1	R2
145.3	165.9	170.9

## TUBER WIDTH (mm):

## AVERAGE:

VARIETY	R1	R2
61.4	68.9	70.9

## RANGE:

VARIETY	R1	R2
54-75	63-76	63-80

## STANDARD DEVIATION:

VARIETY	R1	R2
5.2	3.7	4.5

## AVERAGE WEIGHT OF SAMPLE TAKEN:

VARIETY	R1	R2
145.3	165.9	170.9

## TUBER THICKNESS (mm):

## AVERAGE:

VARIETY	R1	R2
53.8	54.1	57.4

## RANGE:

VARIETY	R1	R2
42-63	45-60	50-68

## STANDARD DEVIATION:

VARIETY	R1	R2
5.0	3.5	4.7

## AVERAGE WEIGHT OF SAMPLE TAKEN:

VARIETY	R1	R2
145.3	165.9	170.9

## TUBER EYE DEPTH:

1 = Protruding; 2 = Shallow; 3 = Intermediate; 4 = Deep; 5 = Very Deep

VARIETY	R1	R2
2	3-4	5

## TUBER LATERAL EYES:

1 = Protruding; 2 = Shallow; 3 = Intermediate; 4 = Deep; 5 Very deep

VARIETY	R1	R2
2-3	3	4

## NUMBER EYE / TUBER:

## AVERAGE:

VARIETY	R1	R2
10.6	12.6	10.8

## RANGE:

VARIETY	R1	R2
9-13	11-13	10-12

## DISTRIBUTION OF TUBER EYES:

1 = Predominantly apical; 2 = Evenly distributed

VARIETY	R1	R2
1	2	2

## PROMINENCE OF TUBER EYEBROWS:

1 = Not prominent; 2 = Slight prominence; 3 = Medium prominence; 4 = very prominence; 5 Other

VARIETY	R1	R2
2	3	3

## PRIMARY TUBER FLESH COLOR: RHSCC

VARIETY	R1	R2
158C	158C	158B

## SECONDARY TUBER FLESH COLOR:

1 = Absent; 2 = Present, please describe

VARIETY	R1	R2
1	1	1

## RHSCC:

VARIETY	R1	R2
—	—	—

## NUMBER OF TUBER / PLANT:

1 = Low (&lt;8); 2 = Medium (8-15); 3 = High(&gt;15)

VARIETY	R1	R2
2	2	2

## 7. DISEASES CHARACTERISTICS:

## DISEASES REACTION:

0 = Not tested; 1 = Resistant; 3 = Moderately resistant; 5 = Moderately susceptible; 7 = Susceptible;

9 = Highly susceptible

## BACTERIAL RING ROT: Foliar reaction

VARIETY	R1	R2
7	7	7

## BACTERIAL RING ROT: Tuber reaction

VARIETY	R1	R2
7	7	7

## LATE BLIGHT

VARIETY	R1	R2
7	7	7

## PLRV

VARIETY	R1	R2
0	0	7

## PVX

VARIETY	R1	R2
0	0	7

## PVY

VARIETY	R1	R2
7	0	7

## OTHER

VARIETY	R1	R2

## OTHER

VARIETY	R1	R2

**8. PESTS CHARACTERISTICS:****PEST REACTION:**

0 = Not tested; 1 = Resistant; 3 = Moderately resistant; 5 = Moderately susceptible; 7 = Susceptible;

9 = Highly susceptible

**GOLDEN NEMATODE**

VARIETY	R1	R2
0		

**OTHER**

VARIETY	R1	R2

**9. GENE TRAITS:****INSERTION OF GENES:**YES ☐NO ☒

IF YES, DESCRIBE

**10. QUALITY CHARACTERISTICS:****CHIEF MARKET:****SPECIFIC GRAVITY:**

1 &lt; 1.060; 2 = 1.060-1.069; 3 = 1.070-1.079; 4 = 1.080-1.089; 5 &gt; 1.090

VARIETY	R1	R2
1	2	1

**TOTAL GLYCOALKALOID CONTENT (mg./100g. fresh tuber):**

VARIETY	R1	R2

**OTHER QUALITY CHARACTERISTICS:****12. CHEMICAL IDENTIFICATION:**

Chemical identification of 'Dakota Rose' was conducted using protein and DNA (SSR) electrophoresis. Chieftain and Red Pontiac were included as standard check cultivars. Protein evaluation was conducted by the North Dakota State Seed Department using the attached protocols. DNA evaluation was conducted by the Department of Plant Sciences, NDSU. Band differences between the two cultivars are shown in Figures 1 and 2. This electrophoretic data allow for the identification of 'Dakota Rose' and show it is uniquely different.

**13. ADDITIONAL COMMENTS AND CHARACTERISTICS:**

See additional information.

## 7.0 LIGHT SPROUT CHARACTERISTICS (+)

## 7.1 Light sprout: general shape

(\*) (+)

		Dakota Rose	Red Pontiac	Chieftain		
spherical	1	4	2	4		
ovoid	2					
conical	3					
broad cylindrical	4					
narrow cylindrical	5					
other (describe)	6					

## 7.2 Light sprout base: pubescence

(\*)

absent	1	9	7	7		
weak	3					
medium	5					
strong	7					
very strong	9					

## 7.3 Light sprout base: anthocyanin colouration

(\*)

green	1	2	2	2		
red-violet	2					
blue-violet	3					
other (describe)	4					

## 7.4 Light sprout base: intensity of anthocyanin colouration (if present)

(\*)

absent	1	7	7	8		
weak	3					
medium	5					
strong	7					
very strong	9					

## 7.5 Light sprout tip: habit

(+)

closed	3	5	5	5		
medium	5					
open	7					



## 7.6 Light sprout tip: pubescence

Dakota Rose Red Pontiac Chieftain

absent	1	5	7	7		
weak	3					
medium	5					
strong	7					
very strong	9					

## 7.7 Light sprout tip: anthocyanin colouration

(\*)

green	1	2	2	2		
red-violet	2					
blue-violet	3					
other (describe)	4					

## 7.8 Light sprout tip: intensity of anthocyanin colouration (if present)

absent	1	7	5	3		
weak	3					
medium	5					
strong	7					
very strong	9					

## 7.9 Light sprout root initials: frequency

low	3	5	5	5		
medium	5					
high	7					

**Additional information for Exhibit C*****Flowers***

Peduncle consists of 15 to 25 buds and branches into two sections. The pedicel articulation is located two-thirds along the length of the pedicel and is purple in color.

*Buds:* Heavily pubescent. *Calyx:* Pubescent. *Corolla:* Red-purple (83C, RHSCC), pentagonal in shape, with a mean diameter of 31 mm. *Anthers:* Five; broad, cone shaped; golden yellow (17B RHSCC) with red pigmented tips; average length is 8 mm.

*Pollen:* Some. Successful hybridizations occur with Dakota Rose used either as the female or male parent. *Stigma:* Capitate; yellow-green (146B, RHSCC). *Berries:*

Production is low under field conditions.

***Tubers***

Tuber set is typically about 8 tubers per plant. *Shape:* Round to oblong and uniform; tubers grown under dryland conditions in North Dakota had a mean length of  $8.23 \text{ cm} \pm 1.24$ , range 5.3 to 10.6 cm; mean width  $6.02 \text{ cm} \pm 0.76$ , range 4.1 to 7.6 cm; mean thickness  $5.30 \text{ cm} \pm 0.72$ , range 3.7 to 8.5 (measured from tubers 51 to 307 g, mean  $149.8 \text{ g} \pm 56.4$ ). *Skin:* Bright red (60B, RHSCC) and smooth textured. *Eyes:* Shallow, number about 10.6 per tuber, and are predominately apically distributed. Eyebrows are slightly prominent. *Flesh:* White (158C, RHSCC). *Dormancy:* Short.

**Glycoalkaloid Levels**

Total glycoalkaloid levels for Dakota Rose are low (Table 1). Dakota Rose averaged 3.37 mg/100 g fresh potato tuber tissue compared to check cultivars, Lenape and Russet Burbank at 18.84 and 7.18, respectively.

TABLE 1. Glycoalkaloid<sup>1</sup> levels for Dakota Rose tubers relative to check genotypes, grown at Absaraka and Wyndmere, ND, in 2004.

Clone	Solanine	Chaconine	Total Glycoalkaloids
	mg/100 g fresh weight basis		
Absaraka			
Dakota Rose	1.07	0.78	1.85
Lenape	7.75	12.55	20.30
Russet Burbank	3.77	3.42	7.18
Wyndmere			
Dakota Rose	2.82	2.06	4.88
Lenape	6.48	10.90	17.38
Russet Burbank	-	-	-

<sup>1</sup> Procedure based on Lafta and Lorenzen (2000), modified for tubers, which does not require heating during extraction of tuber glycoalkaloids.

**Literature Cited**

Lafta, A.M., and J.H. Lorenzen. 2000. Influence of high temperature and reduced irradiance on glycoalkaloid levels in potato leaves. *J Amer Soc Hort Sci* 125:563-566.

EXHIBIT D

#200100227

*Global Agri Services Inc.  
376 New Maryland Highway  
New Maryland, N.B. E3C 1E5  
Tel: (506) 447-8474  
Fax: (506) 454-0567  
Email: globalag@nbnet.nb.ca*

**PBR & PVP TRIAL 2000**  
**UNDINE ROAD, NEW BRUNSWICK**

**OBJECTIVE**

The objective of this experiment is to prepare the potato objective description for the examination of the Plant Breeders' Rights and Plant Variety Protection.

**MATERIALS & METHODS**

**Varieties:**

There was 34 varieties in the trial.

**Design:**

A randomized complete block with 34 varieties and four replicates. All entries were planted in single-row plots and each entry had one plot per replicate. A plot was four rows of 18' long (5.5 m). Spacing between adjacent plots was 36"(91cm).

**Planting:**

The trial was planted on May 19, 2000

**Fertility:**

1500 lbs/A 10-10-10 ( McCain formulation Z)

**Spacing:**

All varieties were planted at 12" (30.5 cm)

**Harvest:**

The field was topkill September 15, 2000 and harvested October 4, 2000

**RAINFALL**

Month of May 21 mm (last week)

Month of June 48 mm

Month of July 104 mm

Month of August 90 mm

Month of September 46 mm

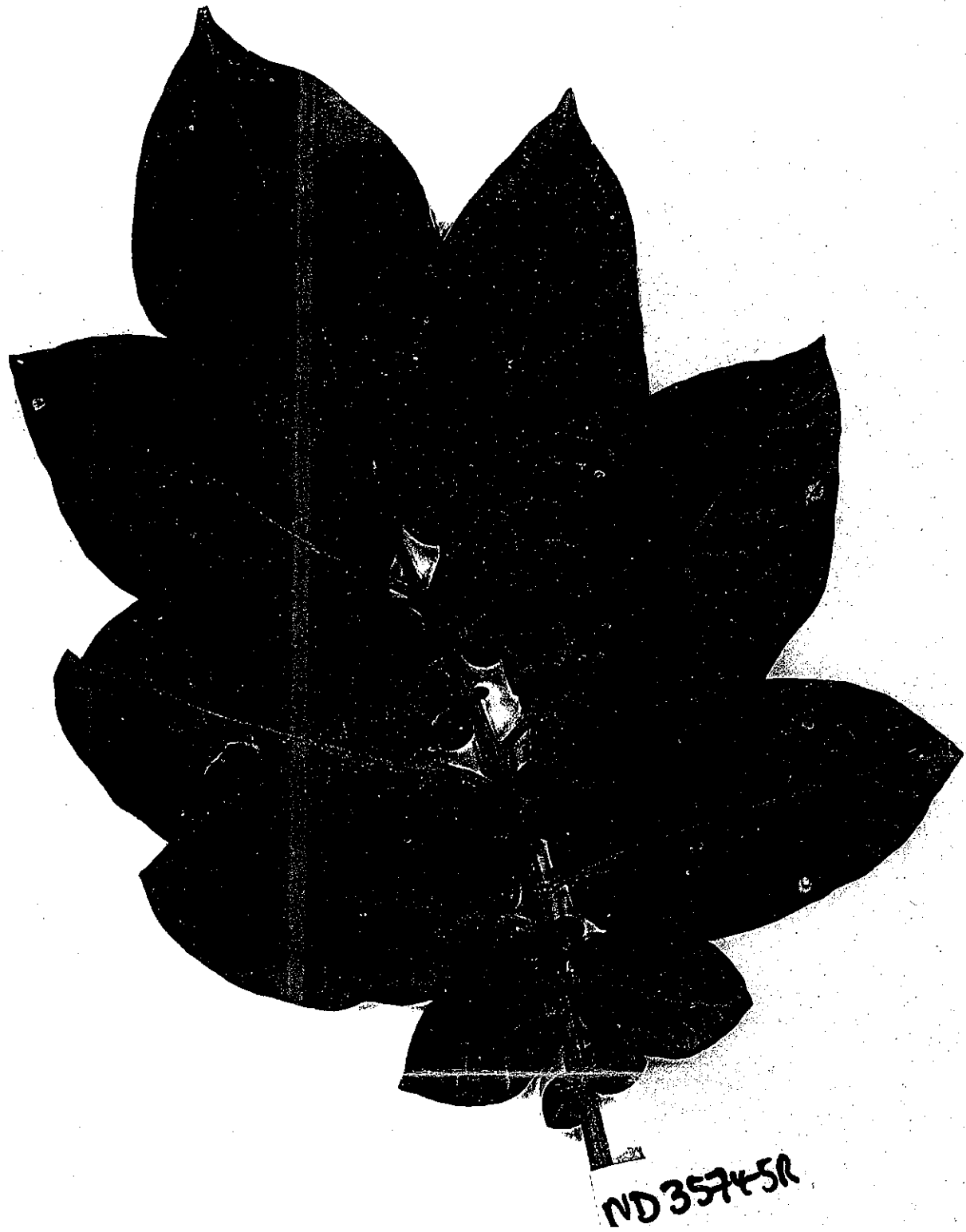
**GENERAL OBSERVATIONS**

The environmental conditions at planting of the trial were normal but cold for this time of the season. The growing season can be summarized with many cloudy days with well disperse rains.

**SECTIONS COMPLETED BY GLOBAL AGRI SERVICES INC.**

1. Market Characteristics
2. Plant Characteristics
3. Stem Characteristics
4. Leaf Characteristics
5. Inflorescence Characteristics
6. Tuber Characteristics
7. Light Sprout Characteristics

#200100227





#200100227

SALE SET UP

CSB  
100

#200100227



Figure 1. Silver stained SSR gels of Dakota Rose and four check cultivars using four SSR primer combinations. Key: 1=Dakota Rose, 2=Red Pontiac, 3=Red Norland, 4=Nordonna, 5=Chieftain.

## SSR Protocol

Genomic DNA was extracted from young leaves according to the method of Fulton et al., 1995 (attached).

SSRs were conducted according to the method of Milbourne et al., 1998, as modified below.

20 ml PCRs consisted of 40 ng genomic DNA, 1x PCR buffer (10 mM Tris-HCl pH 8.3; 1.5 mM MgCl<sub>2</sub>; 50mM KCl), 0.3 U Taq polymerase, 0.15 mmol of forward and reverse primers and 200 mM dNTPs. The cycling conditions for PCR on a MJ Research PTC100 were as follows: 94°C for 2 min, followed by 2 cycles of 94°C for 40 s, T<sub>m</sub> for 30 s, 72°C for 30 s, followed by 28 cycles of 94°C for 25 s, T<sub>m</sub> for 30 s, 72°C for 30 s, followed by 72°C for 5 min. Equal volumes of 95% formamide electrophoresis loading buffer (95% v/v formamide, 10 mM EDTA pH 8.0, 1.0 mg/ml xylene cyanol and bromophenol blue) were added to the samples, which were then denatured at 94°C and snap-cooled on ice. Electrophoresis was carried out on a 33 cm plate in 1 x TBE at 50 to 80W for 1-2.5 hrs (depending on expected product size) on an acrylamide sequencing gel prepared by adding 60 ul of 10% APS and 60 ul TEMED to 50 ml of 5% (w/v) acrylamide; 0.2% bis-acrylamide; 7M Urea; 1x TBE. After electrophoresis gels were fixed for at least 30 min in 7.5% acetic acid, rinsed in water, and silver stained as described below. Primers are listed below in Table 1.

Adapted from <http://www.scri.sari.ac.uk/SSR/potatossrassay.html>

Table 1. SSR primers and annealing temperatures used (primers from Milbourne et al., 1998)

SSR	Repeat	F-Primer	R-Primer	T <sub>m</sub>
STM0038	(TA) <sub>4</sub> (TG) <sub>12</sub>	AACTCTAGCAGTATTTGCTTCA	TTATTTAGCGTCAAATGCATA	54
STM2005	(CTGTTG) <sub>3</sub>	TTTAAGTTCTCAGTTCTGCAGGG	GTCATAACCTTTACCATTGCTGG	60
STM2022	(CAA) <sub>3</sub> ..(CAA) <sub>3</sub>	GCGTCAGCGATTTCAGTACTA	TTCAGTCAACTCCTGTGCG	59
STM3016	(GA) <sub>27</sub>	TCAGAACACCGAATGGAAAAC	GCTCCAACTTACTGGTCAAATCC	60

## Silver Staining Protocol (1 liter solution)

1. Soak Gel in 7.5% Acetic Acid for ≥ 30 minutes (to overnight)
2. Wash 3X in ddH<sub>2</sub>O, 5 minutes each wash.
3. Stain: Silver Nitrate (AgNO<sub>3</sub>) 1 gram/liter, 0.055% formaldehyde, 30 minutes with agitation
4. Wash 1X in ddH<sub>2</sub>O for <10 seconds
5. Add chilled (<10°C) developer: 30 g/l NaCO<sub>3</sub> (Sodium Carbonate), 0.055% formaldehyde, 2 mg/l Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (sodium thiosulfate – must be freshly prepared)
6. As soon as bands appear add 1 liter 7.5% acetic acid stop solution
7. Rinse briefly in ddH<sub>2</sub>O and dry on glass plate in fume hood

#200100227

Citation: Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R.  
1998. Isolation, characterization, and mapping of simple sequence repeat loci in potato.  
Mol. Gen. Genet. 259:233-245.

*Arabidopsis* update

## Arabidopsis Meetings

June saw the 6th International Arabidopsis Meeting at Madison Wisconsin, USA. Over 650 participants enjoyed a varied and action-packed programme of talks and over 500 posters. A full report of the meeting will be given in the December issue of the *Reporter*.

Whilst we are on the topic of arabidopsis meetings, a date for next year's diary: The 7th International Arabidopsis Conference will be held 24-28 June 1996 at the University of East Anglia, which is located right by the Norwich Research Park and the John Innes Centre for Plant Science Research, Norwich, UK. Lecture sessions will be devoted to:

- Floral development
- Vegetative development
- Floral transition and embryogenesis
- Plant pathogen interactions
- Hormones
- Metabolism
- Photoperception

The deadline for registration will be 1 March 1996. Full details of the meeting including the cost of registration will be carried in the next issue of *Weeds World*.

—Mary Anderson

E-mail: PLZMLH@VAX.CCC.NOTTINGHAM.AC.UK  
Nottingham Arabidopsis Stock Centre  
Department of Life Science, University of Nottingham  
University Park, Nottingham NG7 2RD, UK

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TRN # 4480644 Lorenzen, James

*Commentary*

## Microprep Protocol for Extraction of DNA from Tomato and other Herbaceous Plants

Theresa M. Fulton, Julapark Churnwongse, Steven D. Tanksley

Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell  
University, Ithaca, New York 14853, USA

**Key words:** PCR, Southern blots

The extraction of DNA from plant tissue is a critical and often very time-consuming step in many plant molecular biology procedures. This is especially true for studies of molecular genetics, QTLs, or molecular-marker-based breeding where hundreds or even thousands of plant samples need to be analyzed in a short period of time. Many protocols are laborious and are limited by the need for large amounts of plant tissue.

Based on the methods originally described by Murray et al. (1980), we have developed a procedure in our laboratory that maximizes the number of plant samples one person can extract and that yields sufficient DNA for 50 to 100 PCR reactions or two to four Southern blots. The use of very small, new leaves makes it possible to extract DNA from seedlings only one to three weeks old, reducing the need for large amounts of greenhouse space. The entire procedure can be done in 1.5-ml microcentrifuge tubes, eliminating the need for large centrifuges. Using this procedure, one person can isolate DNA from several hundred plants per day.

### Materials and Solutions

**Drill:** Heavy duty household drill with keyless chuck (so pestles can be easily replaced) and plastic drill bit/pestles (VWR Scientific, catalog no. KT95050-99).

**Microcentrifuge:** with a fixed-angle rotor, capable of 10,000 rpm and holding as many samples as possible.

**Abbreviation:** PCR, polymerase chain reaction.

27 DNA extraction buffer: 0.35 M sorbitol, 0.1 M tris-base, 5 mM EDTA, pH 7.5.

28 Nuclei lysis buffer: 0.2 M tris, 0.05 M EDTA, 2 M NaCl, 2 % CTAB, Sarkosyl, 5 % w/v.

29 Microprep buffer: 2.5 parts DNA extraction buffer, 2.5 parts nuclei lysis buffer, 1.0 part 5% Sarkosyl. Add 0.3 to 0.5 g sodium bisulfite/100 mL buffer immediately before use (can be increased to avoid color in final product).

30 For 75 extractions: 25 mL DNA extraction buffer; 25 mL nuclei lysis buffer; 10 mL Sarkosyl; 60 mL microprep buffer; add 0.2 g sodium bisulfite.

## Protocol

- \* Collect 50-100 mg of leaf tissue (approximately 4-8 new leaflets, up to 1.5 cm long) from a 1- to 3-week-old tomato seedling<sup>1</sup> and nestle loosely in the bottom of a 1.5 mL Eppendorf tube.<sup>2</sup>
- \* Prepare fresh microprep buffer (see recipe below); keep at room temperature.
- \* Add 200  $\mu$ L of buffer and grind tissue with power drill and plastic bit, rinsing pestle with water between samples; add another 550  $\mu$ L of buffer and either vortex lightly or shake entire rack by hand.
- \* Incubate in 65 °C waterbath for 30-120 minutes.
- \* Fill the tube with chloroform:isoamyl (24:1). Mix well. (This can be done by vortexing each tube or sandwiching tubes between two racks and vigorously inverting or shaking up and down 50-100 times).
- \* Centrifuge tubes at 10,000 rpm for 5 minutes.
- \* Pipet off aqueous phase (usually approximately 0.5 mL) into new microfuge tubes. Add 2/3 to 1 times the volume of cold isopropanol to each tube. Invert tubes repeatedly until DNA precipitates.
- \* Immediately spin at 10,000 rpm for 5 minutes (no more), pour off isopropanol and wash pellet with 70% ethanol.<sup>3</sup>
- \* Dry pellet by leaving tubes upside down on paper towels for approximately 1 h or placing on sides in seed dryer for 15 minutes (longer if necessary).
- \* Resuspend DNA in 50  $\mu$ L of TE at 65 °C for 15 min.
- \* Spin 10 min at 10,000 rpm, store at 4 °C for up to 1 week or -20 °C for longer storage.
- \* For RFLP use, digest 15 to 25  $\mu$ L for one Southern blot (can expect 5-10  $\mu$ g DNA, use 15-20 units of enzyme). For PCR, use 1  $\mu$ L.

## Notes

1. If only PCR is needed, one can use as little as 1 cotyledon resuspended in 50  $\mu$ L of TE in the resuspension step, but use 5  $\mu$ L to PCR.
2. Tissue can be harvested and kept at room temperature for up to 3 hours or stored at 4 °C for up to 3 days.

Troubleshooting	
Problem	Possible solution
Low concentration of DNA	Grind longer. Use younger tissue.
DNA does not form firm pellet	After spin, replace 500 $\mu$ L isopropanol with 70 % v/v EtOH; gently mix; re-spin.
DNA does not digest	Dry pellet longer before resuspension (remove alcohol residue).
DNA does not PCR	Take only the aqueous layer of the chloroform gradient; do not take any interface, and avoid chloroform residue. Spin for shorter time after isopropanol precipitation; avoid spinning down starch, etc. See solutions for not digesting. Use different amount of DNA (probably less).

3. Can stop here, storing pellet in 70% v/v EtOH at -20 °C indefinitely.

## Comments

Yield should be approximately 10 to 20  $\mu$ g of DNA per plant, enough for two to four Southern or 50 to 100 PCR reactions. The number of samples can be maximized by using two drills with drill stands concurrently and an on/off switch operated by a foot pedal. Because the procedure can be used on very young seedlings, minimal time is wasted waiting for plants to grow. Since one person can extract DNA from several hundred seedlings in one day, whole populations can be processed quickly, and results from PCRs or Southern blots can be available in a few days. This protocol is known to work on tomato, pepper, potato, apple, tobacco, strawberry, *Arabidopsis*, and artichoke.

**Acknowledgments:** Research in our laboratory is supported in part by grants from the National Research Initiative Cooperative Grants Program, Plant Genome Program USDA (No. 58-1908-5-001) and by the Binational Agricultural Research and Development Fund (No. US-2427-94).

## Reference

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8:4321-4325.

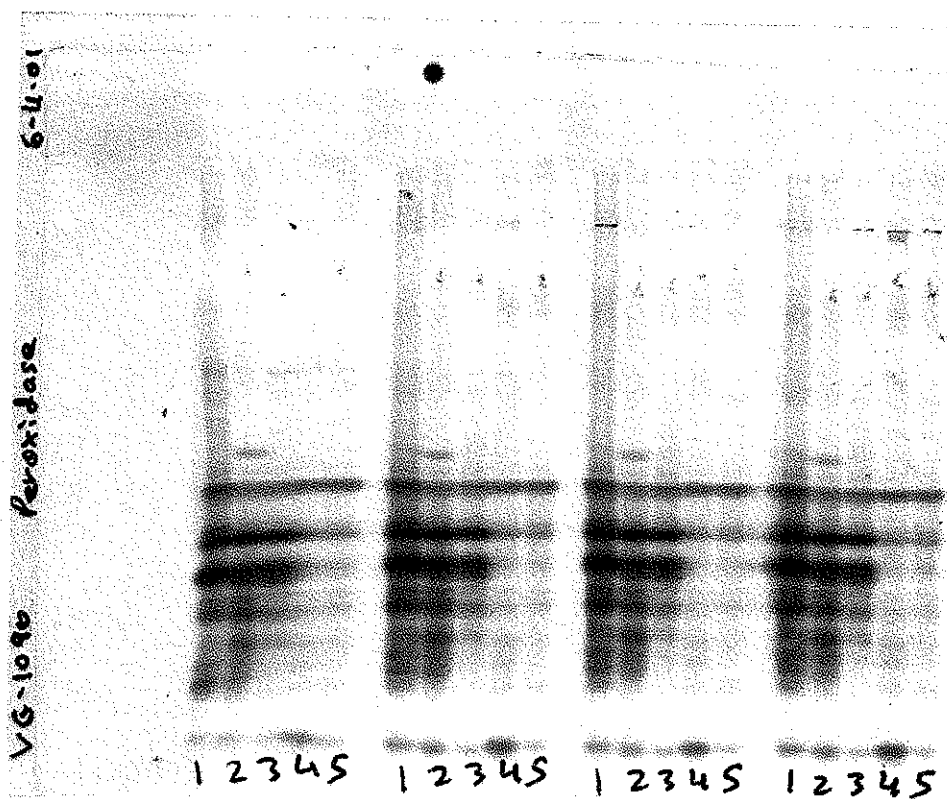
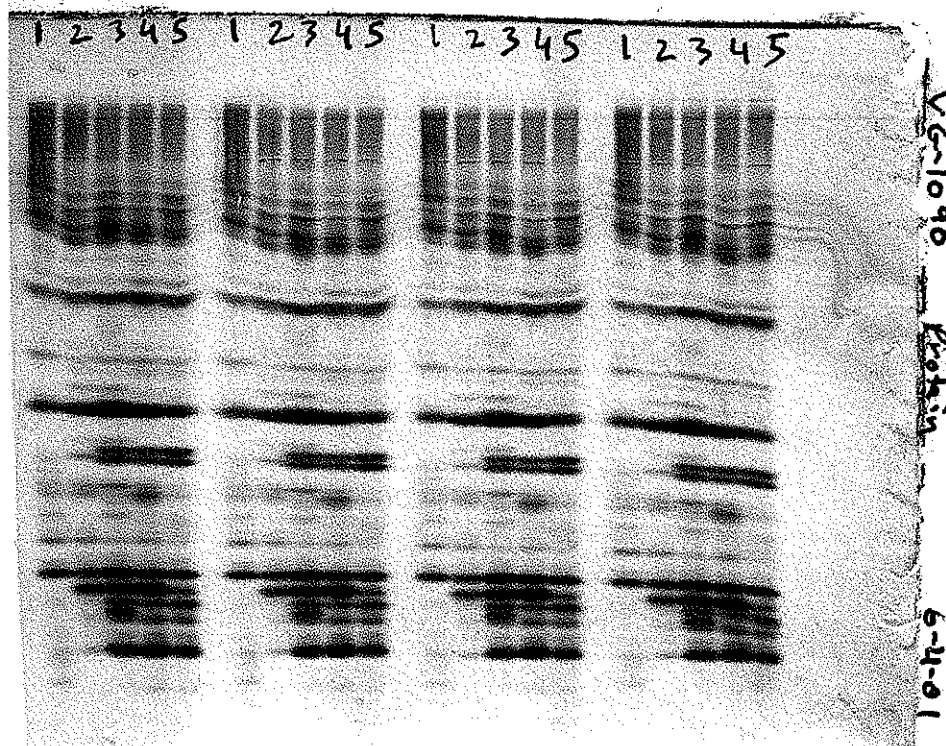


Figure 2. Protein gels of Dakota Rose and four check cultivars using protein stain (blue) and peroxidase staining (orange-red). Key: 1=Red Norland, 2=Nordonna, 3=Dakota Rose, 4=Chieftain, 5=Red Pontiac.

**Procedure  
for the  
Identification of Potato Tubers  
Using Isoelectric Focusing  
and Enzyme Staining**



## Materials Needed

### Equipment Available from Isolab:

Pharmacia LKB Multiphor II Horizontal Electrophoresis Unit (Code 2117-003)  
2000 Volt Power Supply  
Circulating Water Bath (Code RTE-110A)  
Third Electrode for Multiphor II (Code 2117-322)  
Laboratory Rocking Platform (Code 55-D)  
Staining Trays (Code FR-9002)  
Blotting Paper (Code FS-5082)  
PreCut IEF Electrode Wicks (Code FR-9081)  
Gel Dryer (Code FR-9600) - optional  
Sample Templates-Plastic 36 well (Code FS-5036) or  
Sample Templates-Plastic 52 well (Code VG-5048)  
HyPure Gel VG-1090  
HyPure Gel FS-5080

### Additional Laboratory Supplies

Microsyringes or Micropipetors  
Beakers  
Stir Bars and Stir Plate  
pH Meter  
1.5 mL Microcentrifuge Tubes or Microtiter Plates  
Microcentrifuge  
Vortex  
Spatulas  
Timer

Chemicals

Buffers

Sodium Phosphate (monobasic)  
Sodium Acetate  
Sodium Citrate

Sigma Numbers

No. S-0751  
No. S-8750  
No. S-4641

Staining Salts. Co-Factors

Fast Blue RR Salt  
Magnesium chloride  
Ortho-dianisidine  
Fast Black K Salt

No. F-0500  
No. M-0250  
No. D-3252  
No. F-7253

Substrates

$\alpha$ -naphthyl acetate  
1-naphthyl phosphate

No. N-8505  
Aldrich No. 85,541-3

Miscellaneous

Ethanol 95% Reagent Grade  
Glacial Acetic Acid  
Sodium Hydroxide  
Acetone  
Polyethylene Glycol (MW 400)  
Hydrogen Peroxide (3%)  
Methanol (>99%)  
JB-2 Staining System (Peroxidase)

Isolab No. FR-9460 -optional

## PREPARATION OF SOLUTIONS

Mix each of these solutions in a beaker using a magnetic stir bar.

### 0.6 M Sodium phosphate (pH 6.1)

Add 71.9 g sodium phosphate (monobasic) to 900 mL of distilled or deionized water. Titrate with 6.0 M NaOH to adjust solution to pH 6.1. Dilute to 1 L with distilled or deionized water.

2.1 g NaOH  
14.4 g NaOH  
3.0 g NaOH  
20.0 g NaOH

### 0.1 M Sodium acetate (pH 5.0)

Add 8.2 g of Sodium Acetate to 900 mL of distilled or deionized water. Titrate with Glacial Acetic Acid to adjust solution to pH 5.0. Dilute to 1 L with distilled or deionized water.

200 mL  
1.8 g NaAc  
500 mL H<sub>2</sub>O

### 6 M Sodium Hydroxide - to be used to titrate solutions

Add 24 g Sodium Hydroxide to 90 mL of distilled or deionized water. Stir until dissolved. Dilute to 100 mL with distilled or deionized water.

### 0.5 M Citrate Buffer (pH 4.4)

Add 14.7 g of Sodium Citrate to 90 mL of distilled or deionized water. Titrate with concentrated HCl to adjust pH to 4.4. Dilute to 100 mL with distilled or deionized water.

### Anolyte Solution

0.5 M Acetic Acid - Add 15 mL Glacial Acetic to 485 mL of distilled or deionized water.

### Catholyte Solution

0.5 M Sodium Hydroxide - Add 10 g Sodium Hydroxide to a beaker, bring volume up to 500 mL with distilled or deionized water. Stir until Sodium Hydroxide is dissolved.

### PEG Wash Solution

Add 250 mL Polyethylene Glycol (MW 400) to 750 mL distilled or deionized water, stir gently.

### Enzyme Stop Solution

Add 80 mL Glacial Acetic Acid and 250 mL 95% Ethanol to 670 mL of distilled or deionized water, stir gently.

## Sample Preparation of the Potato Tuber

Individual potato tubers are peeled and a section is cubed and placed into a 1.5 mL microcentrifuge tube. (Do not use the outer skin of the potato.)

Freeze the samples at 0°C for at least 24 hours. Thaw the samples when ready for use.

Centrifuge the samples for 3 minutes at 10,000 G in a microcentrifuge.

We use the native juice from the tuber as the sample material.

Analyze the resulting supernatant immediately after centrifugation.

## VG-1090

### Electrofocusing of Samples

Turn on circulating water bath and set temperatures to 15°C.

Locate the STAND BY switch on the back of the power supply and turn it ON at least 15 minutes prior to the run. Level the electrophoresis unit by placing the spirit level on the cooling plate and adjusting the leveling feet on the bottom of the electrophoresis unit.

Clean the cooling plate with water and towel dry.

Remove a HyPure Gel VG-1090 from its package. Carefully remove the protective cover sheet from the gel surface. Do not touch the gel or use it if it is torn or dried. Cut the bottom right hand corner of the gel; this corner will be used as a reference point.

Pipet 3 mL of water onto the center of the Multiphor II cooling plate. Hold the gel by its diagonal corners to form a parabola. Place the bottom of the parabola on the bead of water and slowly distribute the bead of water by using the backing of the gel. Be careful not to trap air bubbles between the gel and cooling plate.

Center the gel on the Multiphor II cooling plate.

Thoroughly blot any excess water from the periphery of the gel using a paper towel.

Place a piece of Blotting Paper evenly on the gel. Smooth the paper lightly with fingertips. After five seconds, gently remove the blotting paper.

Prepare three Precut IEF Wicks. (Two of the wicks will be used as anode wicks, one will be used as the cathode wick.)

Place the two "anode" wicks, rough-side down on several white paper towels.

**NOTE:** Brown or colored paper towels may exude a dye into the electrode wick. Use white paper towels only.

Evenly saturate each wick with 4-5 mL of Anolyte Solution. Gently blot the wicks to rid excess fluid. The wicks should just begin to appear dry before being placed on the gel.

Potato Tuber 01

Place each anode wick onto the gel. Run a finger along the entire length of the wick to ensure even contact between the wick and gel. Refer to the Alignment Grid Diagram for proper placement of the wicks.

Wash hands of residual Anolyte Solution. Place the remaining wick, rough-side-down, on several white paper towels. Evenly saturate the wick with 4-5 mL of Catholyte Solution. Gently blot the wick with another paper towel to rid any excess fluid (wick should just begin to appear dry). Place the wick along the center of the gel between the two anode wicks. Make sure all the wicks are parallel to each other. Run a finger along the entire length of the wick to ensure even contact between the wick and gel.

Position the templates as close to the anode wicks as possible when staining for Peroxidase.

Position the templates 2 cm from the cathode wick when staining for Acid Phosphatase.

Apply 10  $\mu$ L of sample supernatant to each sample well.

Place the electrode cover temporarily into the dimples on the Multiphor II frame and slide the moveable electrodes over the respective anode and cathode wicks. Center the electrodes evenly over the wicks before lowering the electrode cover.

Connect the electrode leads to the connectors on the Multiphor II. The two anode electrodes (red) should be at the top and bottom of the gel and the cathode electrode (black) should be along the center of the gel.

Place the Multiphor II safety cover over the unit and connect the leads from the lid into the power supply. Red lead to the red (+) outlet on the power supply and black lead to the black (-) outlet.

Run the HyPure gel at Constant Power and limit the Voltage to 1500.

Turn the Power Supply "ON." Run the gel for 60 minutes at 40 Watts, then turn the Power Supply "Off."

Remove the clear Multiphor II lid, disconnect the electrodes and remove electrode cover. Remove the templates. Blot each wick and the gel periphery with a white paper towel. Wipe the electrode cover of any condensation that may have developed.

Potato Tuber 01

Replace electrode cover, reconnect electrodes to base unit and place clear Multiphor II lid over the unit. Turn Power Supply "ON." Turn the Adjustment knob until the Watts meter shows 60 WATTS. Electrophorese the gel an additional 20 minutes at ~~60~~<sup>70</sup> WATTS.

When the electrophoresis run is complete, turn the power supply "Off." Remove the Multiphor safety cover and electrode cover. Remove the wicks from the gel's surface.

## FS-5080

### Electrofocusing of Samples

Turn on circulating water bath and set temperatures to 15°C.

Locate the STAND BY switch on the back of the power supply and turn it ON at least 15 minutes prior to the run. Level the electrophoresis unit by placing the spirit level on the cooling plate and adjusting the leveling feet on the bottom of the electrophoresis unit.

Clean the cooling plate with water and towel dry.

Remove a HyPure Gel FS-5080 from its package. Carefully remove the protective cover sheet from the gel surface. Do not touch the gel or use it if it is torn or dried. Cut the bottom right hand corner of the gel; this corner will be used as a reference point.

Pipet 3 mL of water onto the center of the Multiphor II cooling plate. Hold the gel by its diagonal corners to form a parabola. Place the bottom of the parabola on the bead of water and slowly distribute the bead of water by using the backing of the gel. Be careful not to trap air bubbles between the gel and cooling plate.

Center the gel on the Multiphor II cooling plate.

Thoroughly blot any excess water from the periphery of the gel using a paper towel.

Place a piece of Blotting Paper evenly on the gel. Smooth the paper lightly with fingertips. After five seconds, gently remove the blotting paper.

Prepare three Precut IEF Wicks. (Two of the wicks will be used as anode wicks, one will be used as the cathode wick.)

Place the two "anode" wicks, rough-side down on several white paper towels.

**NOTE:** Brown or colored paper towels may exude a dye into the electrode wick. Use white paper towels only.

Evenly saturate each wick with 4-5 mL of Anolyte Solution. Gently blot the wicks to rid excess fluid. The wicks should just begin to appear dry before being placed on the gel.

Potato Tuber 01



Place each anode wick onto the gel. Run a finger along the entire length of the wick to ensure even contact between the wick and gel. Refer to the Alignment Grid Diagram for proper placement of the wicks.

Wash hands of residual Anolyte Solution. Place the remaining wick, rough-side-down, on several white paper towels. Evenly saturate the wick with 4-5 mL of Catholyte Solution. Gently blot the wick with another paper towel to rid any excess fluid (wick should just begin to appear dry). Place the wick along the center of the gel between the two anode wicks. Make sure all the wicks are parallel to each other. Run a finger along the entire length of the wick to ensure even contact between the wick and gel.

Position the templates 2 cm from the cathode wick.

Apply 10  $\mu$ L of sample supernatant to each sample well.

Place the electrode cover temporarily into the dimples on the Multiphor II frame and slide the moveable electrodes over the respective anode and cathode wicks. Center the electrodes evenly over the wicks before lowering the electrode cover.

Connect the electrode leads to the connectors on the Multiphor II. The two anode electrodes (red) should be at the top and bottom of the gel and the cathode electrode (black) should be along the center of the gel.

Place the Multiphor II safety cover over the unit and connect the leads from the lid into the power supply. Red lead to the red (+) outlet on the power supply and black lead to the black (-) outlet.

Run the HyPure gel at Constant Power and limit the Voltage to 1500.

Turn the Power Supply "ON." Run the gel for 60 minutes at 35 Watts, then turn the Power Supply "Off."

Remove the clear Multiphor II lid, disconnect the electrodes and remove electrode cover. Remove the templates. Blot each wick and the gel periphery with a white paper towel. Wipe the electrode cover of any condensation that may have developed.

Potato Tuber 01

Replace electrode cover, reconnect electrodes to base unit and place clear Multiphor II lid over the unit. Turn Power Supply "ON." Turn the Adjustment knob until the Watts meter shows 60 WATTS. Electrophorese the gel an additional 20 minutes at 60 WATTS.

When the electrophoresis run is complete, turn the power supply "Off." Remove the Multiphor safety cover and electrode cover. Remove the wicks from the gel's surface.

## Staining of Samples

### Esterase - EST

## FS-5080

When focusing is complete, immediately pour on stain.

#### To Prepare Stain:

Thirty minutes before staining prepare a 10 mL solution of 50% Acetone and 200 mg of  $\alpha$ -naphthyl acetate.

Five minutes before staining add 200 mg Fast Blue RR Salt to 200 mL 0.6 M Phosphate Buffer (pH 6.1), stir vigorously.

Just before staining add previously prepared acetone solution above and stir.

**NOTE:** Chemicals do not fully dissolve.

Pour stain over the gel surface immediately after focusing. It is helpful to rock or agitate the gel while staining. Remove any air bubbles by gently lifting the gel corners with a spatula. Allow the staining to continue for 20-40 minutes or until the bands reach strong intensities. Stop the enzymatic reaction with 200 mL Enzyme Stop Solution for 3 minutes. Rinse the gel with deionized water for one hour. Gels can be dried in a GelDryer (Isolab FR-9600) for approximately two hours or overnight at room temperature (15°C-30°C). Stained patterns on gels do not fade when dried. The gel can now be analyzed and interpreted. Gently wipe the front and back of the dried gel with a Kimwipe<sup>®</sup> or other lintless laboratory tissue that has been wet with Methanol; this will remove any residual stain.

## Staining of Samples

### Peroxidase - PER

## VG-1090

Immediately after the focusing is complete, place the gel into a staining tray, agarose side up. Pour the Peroxidase Stain Solution onto the gel.

#### To Prepare Stain:

In a 250 mL beaker, with a magnetic stir bar add:

- 40 mL Ortho-dianisidine stain (JB-2 Staining System Bottle A)
- 20 mL citrate Buffer (pH 4.4) (JB-2 Staining System Bottle B)
- 140 mL distilled or deionized water

#### Just before staining add:

- 10 mL 3% Hydrogen Peroxide (JB-2 Staining System Bottle C)

Place the beaker on a stir plate and stir for 5 seconds. Immediately pour the stain onto the focused gel. It is helpful to rock or agitate the gel while staining. Remove any air bubbles by gently lifting the gel corners with a spatula. Allow the staining to continue for 15-30 minutes or until the bands reach strong intensities. Rinse the gel with deionized water for one hour. Gels can be dried in a GelDryer (Isolab FR-9600) for approximately two hours or overnight at room temperature (15°C-30°C). Stained patterns on gels do not fade when dried. The gel can now be analyzed and interpreted.

## Staining of Samples

### Acid Phosphatase - ACP

## VG-1090

When focusing is complete, wash the gel for 10 minutes in 200 mL PEG Wash Solution. Discard this solution and immediately pour on stain.

To Prepare Stain:

200 mL 0.1 M Sodium Acetate pH 5.0

Just before staining add:

200 mg Magnesium Chloride  $\cdot 6H_2O$

200 mg Fast Black K Salt

200 mg  $\alpha$ -naphthyl Acid Phosphate

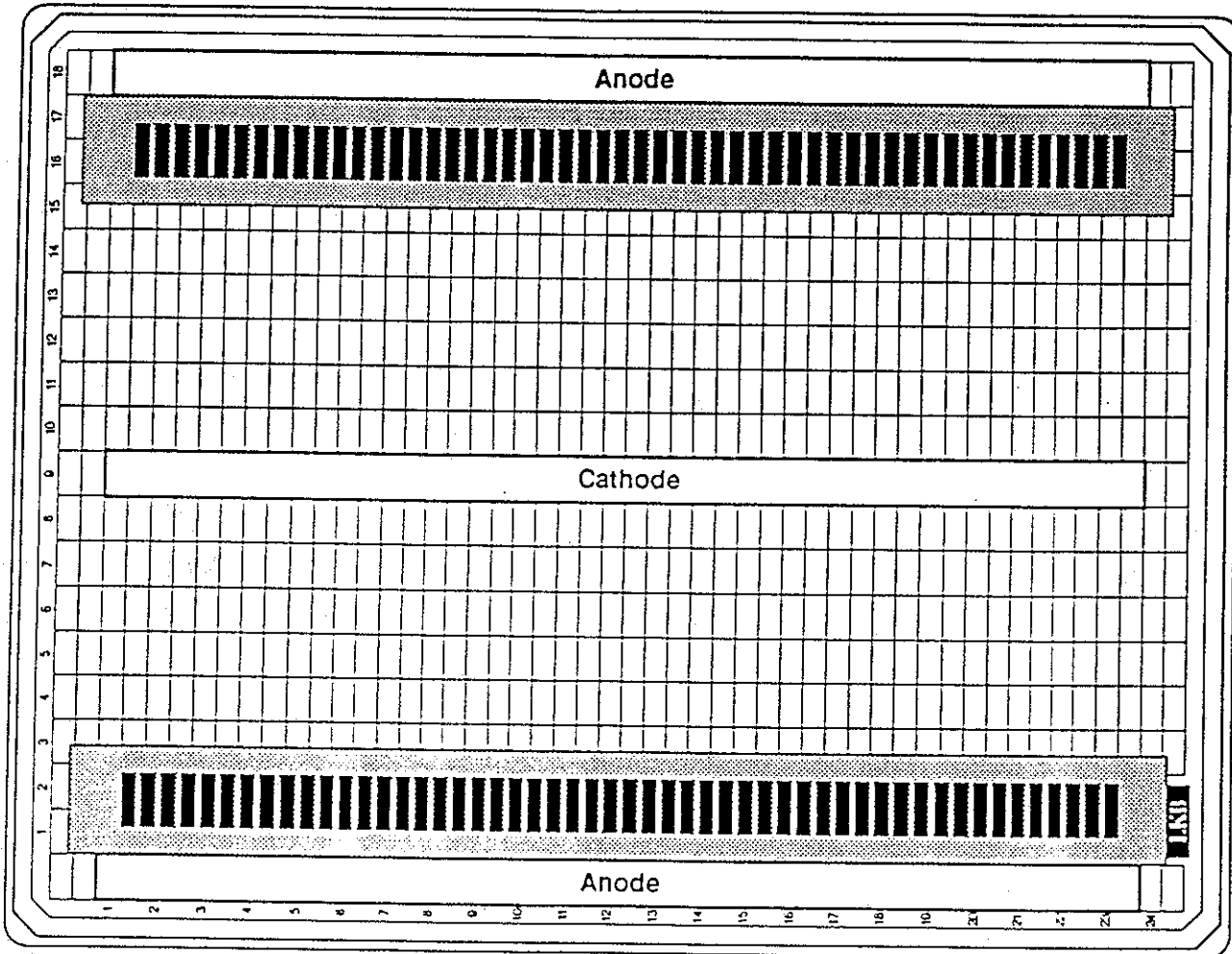
100 mL  
0.8% NAC  
25% HAc  
(100 mL)

200 mL  
1.8% NAC  
25% HAc  
(100 mL)  
+ H<sub>2</sub>O

Stir until all chemicals are dissolved. Pour stain over the gel surface immediately after focusing. It is helpful to rock or agitate the gel while staining. Remove any air bubbles by gently lifting the gel corners with a spatula. Allow the staining to continue for 20-40 minutes or until the bands reach strong intensities. Stop the enzymatic reaction with 200 mL Enzyme Stop Solution for 3 minutes. Rinse the gel with deionized water for one hour. Gels can be dried in a GelDryer (Isolab FR-9600) for approximately two hours or overnight at room temperature (15°C-30°C). Stained patterns on gels do not fade when dried. The gel can now be analyzed and interpreted.

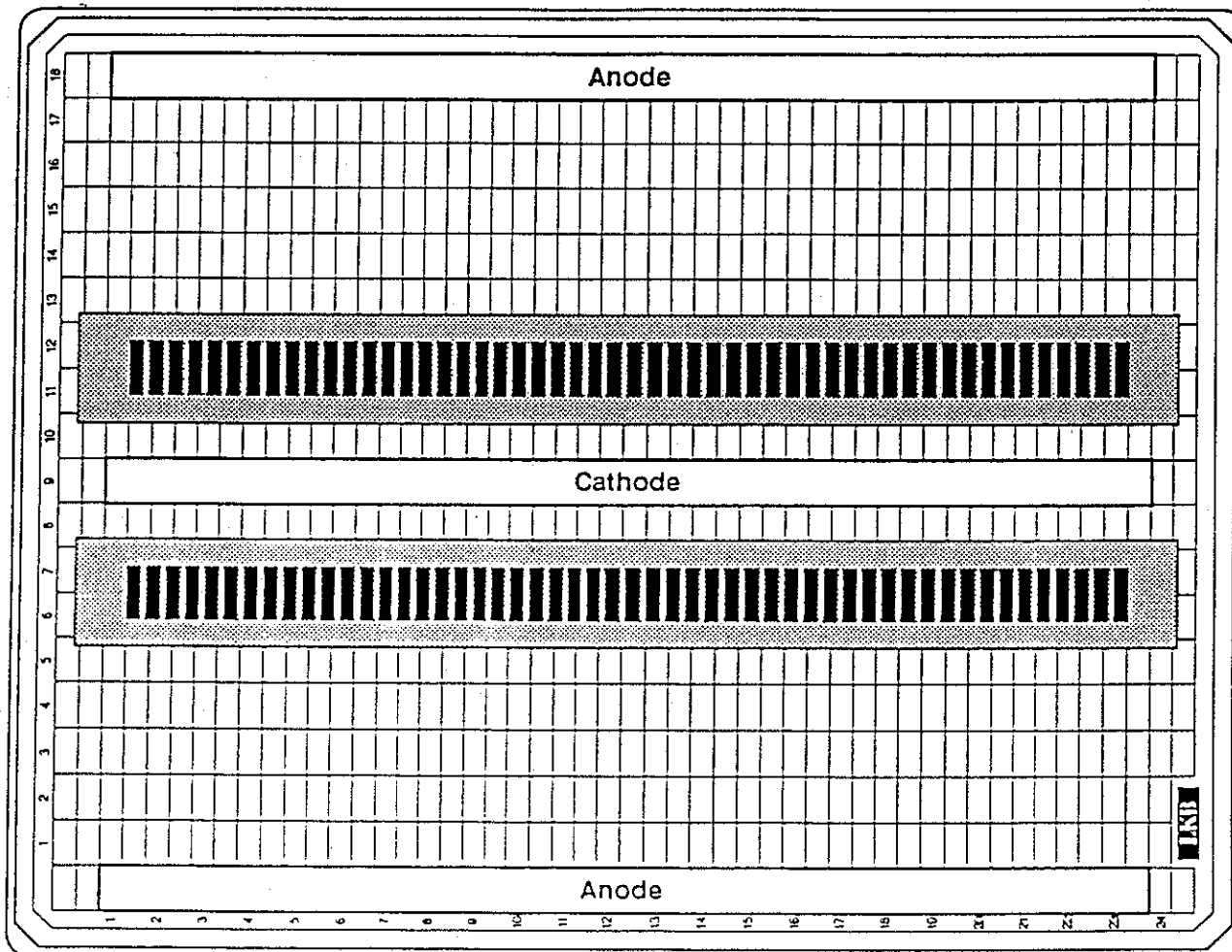


When staining for the enzyme Peroxidase, place templates as close to the anode wicks as possible.



Potato Tuber 01

When staining for the enzymes Esterase or Acid Phosphatase, place templates 2 cm from the cathode wick.



Potato Tuber 01

**Variety Release  
Meeting  
ND 3574-5R**

#200100227



**November 17, 2000**



Summary of ND3574-5R and ND5084-3R's performances relative to other  
cultivars in North Dakota Trials 1993-2000.

Dryland	1993		1994		1995*		1996*	
	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1
Clone								
3574-5R	191	87	188	78	60	86	37	57
5084-3R					73	79		
Red Pontiac	146	76	176	83	95	89	68	76
Red Norland	116	78	152	69	55	75	16	44

Dryland	1997		1998		1999		2000	
	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1
Clone								
3574-5R	120	70	257	92	180	94	172	81
5084-3R	159	86	254	91	284	95	235	91
Red Pontiac	182	80	241	83	362	92	204	93
Red Norland	75	59	193	91	230	88	157	85

\*Flood Year

Summary of ND3574-5R and ND5084-3R's performances relative to other  
cultivars in North Dakota Trials 1993-2000 (Con'd).

Irrigated	1994		1995		1996		1997	
	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1
3574-5R	334	94	384	94	373	93	363	92
5084-3R							411	91
Red Pontiac	429	94	374	95	419	92	413	88
Red Norland	408	93	359	92	329	88	314	87

Irrigated	1998		1999		2000	
	US #1 Yld	% US #1	US #1 Yld	% US #1	US #1 Yld	% US #1
3574-5R	232	88	210	86	257	88
5084-3R	375	91	289	91	298	91
Red Pontiac	382	91	347	85	327	86
Red Norland	200	88	212	77	317	88

#200100227

Summary of ND3574-5R and ND5084-3R's performances relative to other cultivars in North Dakota Trials 1993-2000.

Dryland	11 Trials--8 Years		6 Trials--Last 4 Years	
Clone	US #1 Yld	% US #1	US #1 Yld	% US #1
3574-5R	154	73	170	81
5084-3R			221	90
Red Pontiac	182	84	229	87
Red Norland	122	74	122	78

Irrigated	14 Trials--7 Years		8 Trials--Last 4 Years	
Clone	US #1 Yld	% US #1	US #1 Yld	% US #1
3574-5R	304	91	266	89
5084-3R			343	91
Red Pontiac	388	90	367	88
Red Norland	309	88	261	85

Combined Dryland and Irrigation Last 4 Years			
Clone	US #1 Yld	% US #1	
3574-5R	225	86	
5084-3R	291	91	
Red Pontiac	308	88	
Red Norland	201	82	

#200100227

**Summary of ND3574-5R and ND5084-3R's Performance  
in 3 Years of Sensory Evaluation--Boiling, Baking & Microwaving.**

Clone	Average of Scores	Average (-) Mealiness	Flavor	Color	Mealiness
3574-5R	68.9	58.5	14.3	35.3	10.4
5084-3R	66.9	57.3	14.0	34.4	9.6
Red Pontiac	74.4	60.9	16.6	35.8	13.5
Red Norland	72.8	60.0	16.6	34.5	12.7

## ND3574-5R (ND1196-2R X NorDonna)

### Characteristics

- Good yield potential, equivalent or superior to Red Norland
- High percentage of U.S. No. 1 tubers
- Medium early maturity, slightly later than Red Norland
- Very smooth skinned, slightly oblong tubers
- Bright red skin color
- Retains bright color in storage
- Ranked above Red Norland in North Central Regional Variety Trial
- Seed is commercially available

### Potential Negatives

- Excessive skinning was reported by several growers in 2000 (not noted in previous years)
- One report of storage problems in 2000?

### Disease resistance

- No special resistance noted.
- ? Normal symptoms to PVY, PLRV, ring rot, etc?

#200100227

**EXHIBIT E**  
**STATEMENT OF THE BASIS OF OWNERSHIP**

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF APPLICANT(S)  NDSU Research Foundation	2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER  ND3574-5R	3. VARIETY NAME  'Dakota Rose'
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP, and Country)  1735 NDSU Research Park Drive Fargo, ND 58105-5002	5. TELEPHONE (include area code)  701-231-8931	6. FAX (include area code)  701-231-1013
7. PVPO NUMBER  #200100227		
8. Does the applicant own all rights to the variety? Mark an "X" in appropriate block. If no, please explain. <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		

9. Is the applicant (individual or company) a U.S. national or U.S. based company? ☒ YES ☐ NO  
If no, give name of country

10. Is the applicant the original owner? ☐ YES ☒ NO If no, please answer one of the following:

a. If original rights to variety were owned by individual(s), is (are) the original owner(s) a U.S. national(s)?

☒ YES ☐ NO If no, give name of country

b. If original rights to variety were owned by a company(ies), is(are) the original owner(s) a U.S. based company?

☒ YES ☐ NO If no, give name of country

11. Additional explanation on ownership (if needed, use reverse for extra space):

See additional Exhibit E, Statement of the Basis of the Applicant's Ownership included in this application.

**PLEASE NOTE:**

Plant variety protection can be afforded only to owners (not licensees) who meet one of the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country which affords similar protection to nationals of the U.S. for the same genus and species.
2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.
3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed final breeding. See Section 41(a)(2) of the Plant Variety Protection Act for definition.

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 10 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in its programs on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, and marital or familial status. (Not all prohibited bases apply to all programs). Persons with disabilities who require alternative means for communication of program information (braille, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD).

To file a complaint, write the Secretary of Agriculture, U.S. Department of Agriculture, Washington, D.C. 20250, or call 1-800-245-6340 (voice) or (202) 720-1127 (TDD). USDA is an equal employment opportunity employer.

STD-470-E (07-97) (Destroy previous editions).

Electronic version designed using WordPerfect InForms by USDA-AMS-IMB.

**EXHIBIT E****STATEMENT OF THE BASIS OF THE APPLICANT'S OWNERSHIP**

Drs. Robert H. Johansen (now deceased), Gary A. Secor, James Lorenzen, and Richard Novy are/were employees of the North Dakota Agricultural Experiment Station and North Dakota State University, and are plant breeders who jointly developed Dakota Rose, a red-skinned tablestock potato variety for which Plant Variety Protection is being sought. The employees by agreement and because of the condition of the use of the facilities and funds of the North Dakota Agricultural Experiment Station and North Dakota State University, have assigned all ownership rights for the potato variety Dakota Rose to the North Dakota Agricultural Experiment Station and North Dakota State University.

North Dakota State University on behalf of the North Dakota Agricultural Experiment Station has assigned all ownership of the potato cultivar Dakota Rose to the NDSU Research Foundation. The NDSU Research Foundation is a nonprofit corporation set up to own and manage the intellectual property of North Dakota State University.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 5 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, sexual orientation, marital or family status, political beliefs, parental status, or protected genetic information. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

**U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE  
SCIENCE AND TECHNOLOGY  
PLANT VARIETY PROTECTION OFFICE  
BELTSVILLE, MD 20705**

**EXHIBIT F  
DECLARATION REGARDING DEPOSIT**

NAME OF OWNER (S)  NDSU Research Foundation	ADDRESS (Street and No. or RD No., City, State, and Zip Code and Country)  1735 NDSU Research Park Drive, P.O. Box 5002 Fargo, ND 58105-5002	TEMPORARY OR EXPERIMENTAL DESIGNATION  ND3574-5R
NAME OF OWNER REPRESENTATIVE (S)  Dale Zetocha Executive Director	ADDRESS (Street and No. or RD No., City, State, and Zip Code and Country)  1735 NDSU Research Park Drive, P.O. Box 5002 Fargo, ND 58105-5002	VARIETY NAME 'Dakota Rose' FOR OFFICIAL USE ONLY PVPO NUMBER <b>#200100227</b>

I do hereby declare that during the life of the certificate a viable sample of propagating material of the subject variety will be deposited, and replenished as needed periodically, in a public repository in the United States in accordance with the regulations established by the Plant Variety Protection Office.

Dale Zetocha, Ex. Dir.  
Signature

1/19/07  
Date





**NORTH DAKOTA  
STATE SEED  
DEPARTMENT**

STATE UNIVERSITY STATION  
P.O. BOX 5257  
FARGO, ND 58105-5257  
701-239-7210 FAX: 701-239-7214

#200100227

**RECEIVED BY**

MAY 02 2001

**VICE PRESIDENT FOR  
ACADEMIC AFFAIRS**

April 30, 2001

Ann-Marie Thro, Commissioner  
Plant Protection Office  
AMS, USDA  
Room 500, NAL Building  
10201 Baltimore Boulevard  
Beltsville, MD 20705-2351

Dear Dr. Thro,

This letter is to verify and certify that tissue culture plantlets of the potato cultivar 'Dakota Rose' entered for Plant Variety Protection have been deposited in the North Dakota State Seed Department repository and will continue to be maintained. The facility is a public, state sponsored agency, and cultivars maintained in the repository are available to the general public upon request.

Sincerely,

Ken Bertsch  
ND State Seed Commissioner



North Dakota State  
**NDSSD**  
Seed Department

1313 18<sup>th</sup> St. N., P.O. Box 5257  
Fargo, ND 58105-5257  
Phone: (701) 231-5400  
Fax: (701) 231-5401  
Web: [ndseed.com](http://ndseed.com)

**Ken Bertsch**  
**State Seed Commissioner**

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December 11, 2006

Dr. Paul M. Zankowski, Commissioner  
Plant Protection Office  
AMS, USDA  
Room 500, NAL Building  
10201 Baltimore Boulevard  
Beltsville, MD 20705-2351

Dear Dr. Zankowski,

This letter is to verify and certify that tissue culture plantlets of the potato cultivar 'Dakota Rose' entered for Plant Variety Protection have been deposited in the North Dakota State Seed Department repository and will continue to be maintained. The facility is a public, state sponsored agency, and cultivars maintained in the repository are available to the general public upon request after expiration of the PVP certificate.

The NDSSD Germplasm Repository is located in the potato propagation wing of Johansen Hall on the NDSU campus. *Solanum tuberosum* clones are propagated and maintained by well-established, industry-standard *in vitro* methods as they have been for over 20 years. In addition to normal month-to-month subculturing, a long-term, cool-temperature archival bank is maintained.

Sincerely,

A handwritten signature in black ink, appearing to read "Ken Bertsch", is written over a horizontal line.

Ken Bertsch  
ND State Seed Commissioner

cc. Dale Zetocha